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Studies

from the

Departments

of

Pathology **Bacteriology**

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BEING REPRINTS OF STUDIES ISSUED IN 1915

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NEW YORK

THE HOSPITAL INTERN YEAR AS A REQUIREMENT FOR MEDICAL LICENSURE

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In 1890, I was among those who took the first required course in pathology in the first required third year at the New York College of Physicians and Surgeons. Previous to that time, the medical curriculum consisted of two years of systematic lectures on the five "big" subjects, together with brief discussions of various minor topics. The introduction of a third year permitted the addition of several new laboratory and clinical demonstration courses. We were nervously tried out in a chemical laboratory reviewing many of the test-tube reactions which most of the college graduates had studied under far better auspices as sophomores. Additions were made to the lectures in physics, but this subterfuge did not suppress the indignation which most of the students felt against didactic drilling in this laboratory subject.

In physiology we strained our eyes from a back row of hard benches at half dissected calves, dogs and cats, purporting to demonstrate the mechanisms of the living body. Pharmacologic preparations were lazily passed around the class to the dreary hum of endless didactic lectures compiled from books on materia medica. There were 250 of us and at least one drug for each, but a pharmacologic laboratory demonstration had not been heard of. Many students never attended lectures, and some openly declared it was not worth while. Copies of the lecture notes commanded a ready market.

Not the least depressing influence was the shocking illiteracy of not a few of the lecturers, which was in striking contrast with the dignity, force and careful preparation of a few distinguished men who labored

in vain to teach medicine by precept. The only serious instruction was purchased outside among a group of quiz-masters who had brought the art of cramming to the very zenith of perfection.

The third year had long been talked about, and when it came it was immediately packed full of subjects that had been clamoring for recognition. The class of '91 profited immensely by required courses in physical diagnosis, bedside clinics, obstetric work and close-range demonstrations in some of the specialties. We felt, and the faculty believed, that at graduation we were in a comparative sense real doctors.

But it soon became apparent that if any real knowledge of the medical sciences was to be gained by the student, the didactic lecture had to be replaced by laboratory demonstrations. Bacteriology had been completely overlooked in the new curriculum. This science was regarded as wholly ornamental to the practitioner, but the idea having taken root that the student must know something about the etiology of disease, bacteriology had to be squeezed in somewhere. Finally the clinical departments made more and more urgent demands on the laboratories for systematic help in the laboratory diagnosis of disease, which threatened to convert science departments into mere clinical adjuncts. In 1897, I had the pleasure of organizing a course of clinical microscopy which temporarily satisfied these demands and divested the course in pathology of the honor of analyzing blood, urine and sputum. With increasing efforts to really cover the accepted list of essentials, the curriculum was now full to the bursting point, and the inevitable fourth year was soon added.

With its advent came the first serious effort to train the undergraduate to recognize and treat disease in the living, and the success attending this effort in the last fifteen years has been the most notable achievement in medical education in America. After passing through many phases and experiments, clinical teaching has apparently settled on the system of clinical clerkships in an active hospital as the most efficient method of training practitioners. In adopting the plan, the university medical schools have endeavored to assume full responsibility of making this fourth year a period of systematic instruction and not a trial term of service to a hospital.

During all this long period of the development of clinical instruction, laboratory educators have looked on with approval or given their active cooperation. While recognizing certain defects in the clinical clerk system from the point of view of efficiency and concentration, the prime necessity of making students reasonably competent to recognize and treat disease has outweighed every other consideration.

Yet laboratory teachers have long felt that there is a growing disproportion between the student's real acquaintance with disease and his facility to arrive quickly at a diagnosis of the sick and to apply stated remedies. They have felt that the training of a practitioner is a life work and not a function of curriculums, and they believe that the value of clinical experience after a certain time depends wholly on the breadth of theoretical knowledge of disease. They observe with dismay the contempt which the average graduate shows for anything he cannot see with his eyes, hear with his ears, or do with his fingers.

While watching the remarkable development of many new branches of medical science, as bacteriology, chemical pathology, immunology, and the immense additions to our knowledge of hygiene and preventive medicine, they have said rather little of the genuine demand of these subjects for recognition in the curriculum. They marvel at the scope of such a program as that of the Fifteenth Congress of Hygiene and Demography, while observing that practical medicine is becoming largely a system of therapeutics. They have long cherished the hope that the foregoing and many other subjects which belong in the training of an educated physician might sometime find a place in our educational schemes, but it has been fully recognized that that time can come only when a fifth year is added to the medical curriculum.

It is with astonishment, therefore, that many laboratory and other medical educators have observed the sudden development of a scheme to add a fifth and wholly clinical year to the curriculum without any consideration of the needs of medical education as a whole. It is difficult to say just how this movement originated. It was for a time discussed in the Council on Medical Education of the American Medical Association but was not endorsed by that body. It

has gained much headway in the West, and the state of Pennsylvania has already been induced to adopt the requirement of one year's residence in a hospital as a condition of medical licensure. It is certain that no general discussion of the plan by representative schools of the country has ever taken place. A circular of information issued by the Committee on Pedagogics of the Association of American Medical Colleges in 1914 revealed a curious conglomeration of opinion, including complete endorsement, feeble acquiescence, total unfamiliarity, curt disregard, and forceful disapproval. It is now clear that the adoption of the scheme was hasty and imperfectly considered, but since we are told that the fifth clinical year is coming to stay, it is time to consider carefully its merits and demerits.

I myself believe that the general adoption of a fifth clinical year, as it has been established in certain localities, is not for the best interests of medical education and would create a serious menace from which the American profession would find it difficult to recover.

1. From the educational standpoint it is a tactical blunder for university trustees to relinquish their control of any part of medical education. The states have entrusted to them this function. They have made enormous outlays to meet the responsibility, and the states, unless ill advised, will not repudiate the contract. Yet few schools can provide clinical instruction by their own officers for their own purposes in their own hospitals. Assuming that outside hospitals are of a high order of efficiency, the university school should not turn over to any merely affiliated organization the finishing process in medical training.

2. To place the student at the service of a hospital with definite routine duties to perform in the interests of the hospital is to introduce an unsound educational principle. This difficulty will not appeal to the practical man, but it has been considered by medical educators. The University of Chicago has devised a fifth hospital year as far as possible in the interests of the student, but I fear that even such a carefully guarded practical course cannot be made as effective as a well planned course of genuine advanced university instruction freed from the necessary restraints and accidental interruptions of hospital routine.

3. We have thus far assumed that hospitals are ideal places for conducting medical education; but they are not, and much remains to be done before they can be made such. The hospital is conducted for the benefit of the patient, and it has taken a long campaign to secure the admission of students to most wards. With rare exceptions, the discipline of hospital life is far removed from the careful adjustment of means to ends that dominates university education. On this account, many would admit that a compulsory fifth year should be attempted only in university hospitals; but the adoption of the scheme cannot contemplate any such restriction. It involves consigning some students to some hospitals where the training is quite as likely to harm as to benefit. In Pennsylvania this dilemma has been boldly met by an energetic effort to standardize the hospitals of the state. This is not a small undertaking, and the acknowledgment of its necessity demonstrates the blunder of hasty action in adopting the compulsory clinical year. The hospitals should be made fit before the students are forced into them. It might also appear that much meddlesome interference with the sound development of many struggling institutions, created for local needs, may be exercised by a hospital inspector who arbitrarily demands that every institution receiving interns should provide pathologic laboratories and maternity services. It may very well be better for many institutions to contract for outside pathologic talent, while the best interests of obstetrics are unquestionably served by special obstetric hospitals. The Pennsylvania plan of standardization of hospitals conflicts with the proper development of medical specialties.

4. The surrender of the fifth year to hospitals places undesirable influence in the hands of hospital attendants who cannot qualify for positions on a university faculty. It is not so long since control of a clinical service could purchase a place on a university faculty. It is unnecessary to rehearse the long chapter of medical politics which surrounded the period when prominent hospital attendants controlled medical faculties; but those who have lived through that time do not want to return to the old régime.

5. It is proposed that those students who do not intend to practice medicine may substitute a fifth year in some laboratory. They may do so now, but it is quite clear that if the license to practice depends on a fifth clinical year, it will be much more difficult to induce men to enter laboratory careers. Few students are willing at the outset to abandon the right to practice medicine. The small proportion who might engage in a fifth laboratory year and furnish recruits for the medical sciences will be compelled from a reasonable regard to their natural privileges to decide to take the extra clinical year, after which they will be much less inclined to go back to the laboratory. This highly unfortunate result appears to many laboratory teachers as one of the most serious objections to the compulsory clinical year. Additional difficulties arise if the student desires to pursue postgraduate instruction in another state or another country.

6. It is urged that from 50 to 100 per cent. of the graduates of many schools already take a fifth or a sixth clinical year, so that the requirement of this service will work no hardship. To the same extent it will accomplish no good. More men will take hospital training when their means and time permit and their judgment approves, and when the attractions of hospitals increase; but all the arguments that are raised against prolonging the period of professional training tell with special force against the compulsory practical fifth year.

7. The chief objection to the fifth clinical year lies in the excessive emphasis it places on clinical training and the relative subordination visited on the fundamental sciences and on general medical knowledge. The scheme does not provide for relief for the crowded curriculums of the first three years. It ignores the demands for more thorough instruction in the central sciences of anatomy, chemistry, physiology, bacteriology and pathology. In none of these branches, except perhaps anatomy, are instructors satisfied that students now secure a genuine grasp of the subject. Courses in pathology, especially those limited to the second year, cannot hope to cover adequately the fields of pathologic anatomy, histopathology and pathogenesis. Pathologic anatomy is nearly

a lost art, and although it forms the foundation stone of internal medicine, few internists and fewer surgeons are reasonably familiar with what disease does in the body. The student's glimpse of the immense and significant field of histopathology shortly fades and leaves a void which befogs the practitioner throughout his entire career. Even in the laboratories it is becoming more and more difficult to obtain expert interpretation of histologic changes. The discussion of the pathogenesis of diseases often leads directly into speculation, but the competent medical speculation of today leads to the substantiated body of medical knowledge of tomorrow, and without it medical progress comes to a standstill. Not many medical students become sufficiently acquainted with medical literature to engage successfully in speculation.

The fifth clinical year makes no provision for substantial courses in chemical pathology, immunology, hygiene, preventive medicine, forensic medicine or other specialties, although all these departments have long been waiting patiently for recognition. In some institutions, it is true, courses are given in these subjects, but so far as I can learn, it is at the expense of more important departments.

In other words, the fifth clinical year is planned to develop practical training at the expense of medical knowledge. The issue is clearly before us. Shall the medical school undertake to train practitioners thoroughly or to educate physicians? There could be no clearer demonstration of the necessity of correct principles in the control of educational movements. If the central principle of the medical school is to train practitioners, then the fifth clinical year should be adopted. It will certainly tend to produce that result, and with a modicum of medical knowledge the student will be so familiar with the exigencies of the sick room that he will be able to meet the ordinary demands of practice from the moment of graduation. That he will be able to meet all the demands made of the educated physician is, however, quite unlikely. It is impossible to train physicians in any such sense. They must gain experience from life-long labor, and grow from their own resources.

It is the function of the medical school to turn out broadly educated physicians who are thoroughly

grounded in the knowledge of disease and who will find in this knowledge resources enabling them to profit by their experience as practitioners.

It has been stated that we do not want to train scientists. There is little danger of doing so. We urge the adequate teaching of physiology and pathology because such knowledge makes better practitioners, not because it may make scientists.

It may be highly important to know what the best practitioners are now doing in hospitals, but it is far more important to have some experience in solving a medical problem by one's own resources. The best practice may not always be the present vogue, and improvements in practice do not come from the imitator but from the investigator. I have been assured by many that a fifth year spent in clinical or laboratory research would be of far greater value to the student than a year of routine hospital service. A thorough acquaintance with the literature of one disease or of one aspect of a disease would establish a critical standpoint by which the student could judge the work of his colleagues and the current contributions of the day. A year spent in such work would save the young practitioner from becoming a ready prey to every new medical conceit, and add to his qualifications a competent critical judgment. It is for this reason and not for the object of training scientists that a broad knowledge of the medical sciences is an essential part of the training of practitioners.

Will practitioners as a class, however, consent to be thus summarily excluded from the realm of scientific pursuits? Will they be content meekly to receive orders what to do and report results? Unfortunately this is just the condition in which most practitioners find themselves—resourceless in the face of clinical problems. The pursuit of clinical problems is falling more and more into the hands of laboratory men, and this tendency will be accelerated by any further undue extension of clinical training. If clinicians are not to be scientists, then the progress of medicine must depend on the laboratories while the practitioners treat the sick in the time-honored fashion.

On the contrary, the powerful movement for clinical research in this country indicates that the young practitioners will not consent to be excluded from the

science of medicine. They will not agree that the object of medical education is to train practitioners and not scientists. As long ago as 1846, Philip von Walter wrote, "Woe to that hospital where unscientific men work." The fifth clinical year in the service of a hospital is hardly adapted to the scientific training of clinicians. A fifth year spent on clinical problems with expert assistance would accomplish much more in this direction.

Much depends on one's conception of the function of the medical profession in the community. Those who would limit the activities of the physician to the care of the sick would reduce medicine to a system of therapeutics and even of applied therapeutics. The fifth clinical year commits us to that view, and just here lies its chief danger. As a system of applied therapeutics, medicine comes into competition with the medical cults, with results not always favorable to medicine.

It is a matter of record that when any irregular therapeutic system persistently demands the legal right to practice, it eventually receives its license. Legislatures composed of intelligent laymen are finding it difficult to detect great distinctions between regular and irregular systems of therapeutics and some legislatures have been unable to find any distinction whatever. Hence optometrists and osteopaths are practicing medicine and other cults may soon be doing so. If medicine is to keep out of company with the cults, it must not rest its claims solely on the treatment of the sick, but must meet its increasing responsibilities to medical science. It must turn out educated physicians. It must support the medical sciences by emphasizing their importance to the student choosing a career.

Medical curriculums must give a reasonable place to every department of medical knowledge, and be prepared to receive every new branch of medical activity that presents itself. The plan of introducing a fifth purely clinical year has failed to consider these paramount obligations of medicine, and therefore in its present form it does not accord with the best interests of medicine.

It should be remembered that the fifth year is the last year that can reasonably be added to the medical

course. When it is added, the plan should embrace a readjustment of the entire schedule and a recognition of the demands of many branches of medical knowledge which are now presented inadequately or not at all. The dominant principle in constructing the plan should be the broadest possible education of physicians who may be able to serve the community in any medical capacity, and not alone the training of practitioners to treat the sick.

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ATYPICAL HEMORRHAGIC MALIGNANT HEPATOMA.*

A HISTOLOGICAL STUDY.

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Among the many primary malignant tumors of the liver is a group of multiple, hemorrhagic and largely necrotic growths the nature of which cannot be determined by histological study alone. The chief point of interest in these, as with many other neoplasms, is the question of their histogenesis. Included in this question is also the relation which they bear to the typical primary carcinomas of this organ.

The wide variation in the gross appearance and histological structure of the various reported tumors included in this class has made their study difficult and has led to a great diversity of opinion as to their origin. They have been described as sarcomas, peritheliomas, endotheliomas, and, on account of the peculiar syncytial-like masses and giant cells that have frequently been encountered, they have been considered by some as related to chorioma. Still other observers have maintained their epithelial origin, classing them with carcinomas.

Some of the difficulties of this subject may be revealed by a brief consideration of the characteristics of the primary tumors of the liver. It is not necessary to our purpose to consider all the forms of primary neoplasm of this organ since the present study is limited to those primary new growths in the liver, of either connective tissue or epithelial origin, which exhibit true malignancy.

Sarcoma. — Primary mesoblastic tumors of the liver usually show many types of histological structure and are not easily classified.

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which he argues in favor of its derivation from true cells of the chorion, and since there was in his case the history of a normal pregnancy eighteen months before the death of the patient he considers this fact as evidence that cells of the normal chorion were carried by the circulation and ultimately lodged in the liver, there developing primary chorioma, without any pathological change in the uterus. The possibility of such an origin appears very remote when we consider the entire absence of tumor growth in the lungs and other organs, and also the frequency with which the resemblance to chorioma is observed in hemorrhagic atypical tumors of the liver.

An analogy between the liver tumors of this choriomatous type and teratoid tumors which occur in the testicle and other organs has been suggested (Fischer, Marx). Ewing, 1913, has described two cases of chorioma of the liver secondary to teratoma testis. In both the gross appearance of the hepatic tumor was very similar to an early chorion and the microscopical structure exactly duplicated that of typical chorioma, in which numerous syncytial buds and masses and groups of Langhans cells were conspicuous. In a similar case reported by Warthin the hepatic tumor was composed of the most typical syncytium and Langhans cells. It seems evident, therefore, that when true chorioma occurs in the liver its structure is very characteristic and quite unlike the atypical carcinomas.

Carcinoma. — The primary epithelial tumors of the liver are relatively rare although without doubt more frequently observed than the primary mesoblastic neoplasms. Orth in two hundred and fifty-eight cases of hepatic cancer found primary carcinoma only four times. Goldzieher and Bokay in their statistics give .3 per cent as the ratio, and Eggel in all the literature to 1901 found only one hundred and sixty-two cases. Various attempts have been made to classify epithelial new growths of the liver on a morphological basis alone, but the result has not been entirely satisfactory (Simmonds, 1884, Witwicky, 1899, Wetzold, 1908). We are

indebted to the work of Eggel (1901) for the most complete study of primary carcinoma of the liver, and his classification, namely, two main groups, appears by histological study to be justifiable.

In the first group he places those developed from intrahepatic bile ducts and usually accompanied by cirrhosis (fifty per cent). These growths are circumscribed and often multiple. In the larger nodules there may be areas of necrosis but very seldom hemorrhage. They always show connective tissue stroma and occasionally bile pigment may be demonstrated.

Microscopically they present a glandular structure but the cells are anaplastic and the process is malignant. Epithelial proliferation may destroy the bile duct wall and new ducts may be observed undergoing transformation into cancer alveoli (Eggel, Milne, Winternitz, Mirolubow).

The second group is the so-called "hepatoma," which is derived from the liver parenchyma and occurs as one large mass or several smaller nodules. These tumors in the majority of cases occur with cirrhosis (eighty-three per cent Eggel) and may show the presence of bile pigment. They usually appear late in life, run a short course, and are often mistaken clinically for various forms of cirrhosis.

Microscopically the cells of these hepatomas may resemble hyperplastic liver cells packed closely together without definite arrangement or at times following more or less closely the structure of liver cords. In them several entirely different types of structure may be developed. One may represent a pure adenoma, the "adenoma malignum hepatis" (Blumberg, 1912), which is so often confused with primary carcinoma of bile duct origin. Another may show diffuse carcinoma, or certain areas may resemble perithelioma with several rows of large epithelial cells surrounding dilated blood spaces. Often there are giant cells and large hypertrophic liver cells, which have some of the characteristics of syncytial masses. Goldzieher and Bokay in their cases report transformation of solid trabecular liver cell type into the characteristic gland cell structure with the production of bile.

Finally there will probably always be a few border line neoplasms which may be designated as mixed epithelial tumors of the liver, in which both the bile ducts and liver cells apparently are involved (Milne, Eggel, Beattie and Donaldson, Muir and Rolleston).

In 1907 Ewing reported a case of primary carcinoma of the liver in which wide variation in histological structure was shown in various portions. As this description gives a characteristic picture of many of the atypical hemorrhagic neoplasms of the liver, it seems advisable to quote from his report as follows:

"The clinical history of these cases was apparently rather characteristic in the fact that very few showed any prolonged symptoms, and this particular case was no exception.

"The patient was an old man of sixty-five years, who had had no symptoms of note until the day of his death, which occurred suddenly apparently of abdominal hemorrhage. At autopsy the seat of the hemorrhage was found to be in the right lobe of the liver which was very much enlarged and was the seat of a large softened single tumor, infiltrated with blood and composed of several convoluted nodules measuring about six inches across. No metastases were found in any part of the body.

"Histological study showed three main types of structures in the tumor, each entirely different from the others. First, there were large areas in the tumor which appeared to have developed from a marked increase in number of liver cells with more or less preservation of liver cords. These cords were composed of an increased number of medium-sized cells which were in places associated with huge giant cells. Another characteristic field showed these cords very much increased in width, being composed of six or eight rows of smaller cells separated by cavernous channels.

"A third histological feature in some portions of the tumor was diffuse growth of carcinoma in which the cells were growing irregularly in fibrillated connective tissue which was itself infiltrated with cells, so that in different portions of this tumor one found three totally different structures. In the first only was there any visible relation to the normal liver structure. The transformation of liver cells was rather sudden, that is in the length of five or six cells such a change would be found complete. Another feature of this liver which was of interest was the condition of some foci of the parenchyma in the right lobe. In these portions the liver cells, while preserving their relation to the blood vessels, showed an enormous increase in size without other neoplastic character."

Cruickshank and Teacher, 1910, report a case of primary tumor of the liver with some features of a chorioepithelioma in which in the smallest nodules they found evidence of hyperplastic liver cells undergoing a change which suggested transformation of liver cells into tumor cells. They therefore consider their case one of primary carcinoma of the liver derived from liver parenchyma.

Goldzieher and Bokay (1911) include an atypical primary liver parenchyma carcinoma among the fourteen cases which they have reported. This case showed an unusual arrangement of tumor cells around dilated capillaries, associated with huge giant cells, giving a very characteristic picture of perithelioma. But the cells they considered of a distinct hepatic type and classed the neoplasm as an atypical carcinoma derived from liver parenchyma.

All variations may occur between these distinct types and in one tumor characteristics common to all may be observed or the structure may be so atypical that the histogenesis is uncertain.

It will thus be seen that, in primary tumors of definite epithelial origin, the various atypical structures observed include the picture of perithelioma, endothelioma, angiosarcoma and chorioma.

The writer submits the report of an unusual case of primary hemorrhagic tumor of the liver showing three distinct types of histological structure. In one area the arrangement of cells around blood spaces suggests perithelioma. Another portion shows many of the morphological characteristics of chorioma. Still other parts exactly duplicate the structure of diffuse carcinoma.

This case occurred in the medical ward of the New York Infirmary in the service of Dr. Baldwin, to whom I am indebted for the following clinical history:

Report of case. — "The patient was a married woman seventy-five years old, who had no children and gave no history of a pregnancy. With the exception of a prolonged gastro-intestinal disturbance, she had always enjoyed excellent health. The day before her entrance into the hospital

she became suddenly weak and suffered intense pain in the back and epigastrium, which was accompanied by persistent vomiting and considerable jaundice. The marked increase in the size of the liver led to the diagnosis of hypertrophic cirrhosis. The liver dulness was found to extend from the third interspace to 9 centimeters below the costal margin in the parasternal line. The abdomen was distended, and a mass apparently attached to and continuous with the liver was found; this mass was nodular and on percussion was dull. It measured 25 centimeters at its widest point and the lower margin was 4 centimeters from the umbilicus. The second day after admission to the hospital the patient died suddenly, probably of abdominal hemorrhage.

"POST-MORTEM EXAMINATION. — The body is fairly well nourished, but the skin is deeply jaundiced. The stomach and intestines contain a dark brown fluid resembling changed blood. The liver is immensely enlarged, measuring 30 centimeters across both lobes, and weighing 12 pounds. The surface as well as the transsection of the organ presents a mottled appearance and is studded with numerous nodules of varying sizes, the largest of which are deep red in color, measure 5.5 centimeters x 6 centimeters and project above the surface of the liver. In certain areas the largest hemorrhagic nodules appear to be formed by the coalescence of the smaller growths. The greater number, however, give no evidence of having been formed in this manner. Many of the smaller masses fail to show this hemorrhagic character and are more distinctly necrotic in appearance. Some of these are bile stained, while the smallest present a grayish color. This arrangement of hemorrhagic and necrotic masses of varying sizes closely resembles the characteristic macroscopical picture of a metastatic chorioma in the liver. Although all of these areas are fairly well circumscribed, they do not appear to be surrounded by a distinct fibrous capsule, and are soft and friable in consistence. The mucosa of the gall bladder is free from tumor masses but on the posterior wall, just within the outer coat and not involving the mucosa, is a circumscribed small tumor mass surrounded by a firm capsule. The lymph nodes at the hilum of the liver are greatly enlarged, and on section show tumor tissue identical with that found in the liver. The pancreas is firmly adherent to this mass of lymph nodes but does not show any invasion. The uterus, Fallopian tubes, and ovaries are atrophic.

"The autopsy failed to reveal new growths in any other organs except the lymph nodes at the hilum of the liver, justifying the presumption that we are dealing with a neoplasm having its starting point in the liver itself."

Material from many parts of the liver was hardened in formalin and Müller-formol and sections were stained with hematoxylin and eosin, Van Gieson and Weigert's elastic tissue stain.

The microscopical examination revealed two types in this tumor, namely, necrotic-hemorrhagic and cellular.

The necrotic-hemorrhagic areas are of varying size, the large coalescent ones are composed of a solid blood coagulum with a few scattered cells of large epithelial type with the clear cytoplasm and a very deep staining nuclei. In the smaller hemorrhagic areas are found a few cells lying in rows that bear a faint resemblance to columns of liver cells. While most of these cells are scattered irregularly, their arrangement around blood vessels presents the structure of perithelioma. Many of them resemble hypertrophic liver cells; others are so large that they appear to have been formed by the fusion of several cells. A considerable number contain multiple nuclei forming irregular giant cells resembling the syncytial masses of chorioma.

In all the hemorrhagic areas there is more or less extensive necrosis, and here and there new-formed blood vessels penetrate into these necrotic masses. These young capillaries are surrounded by tumor cells and give a very characteristic peritheliomatous arrangement. Occasionally, however, the necrotic process has advanced so far that there remains the shadowy outline of the vessel only. The arrangement of masses of tumor cells in circumscribed areas, with a more or less definite surrounding wall, conveys the impression of tumor cells developing in a branch of one of the larger vessels distending the vessel until only a faint outline of true wall is left. The presence of elastic tissue and muscle fibers confirms the opinion that it represents the wall of a blood vessel. The fact that tumor cells occur in the capillaries is definitely shown in the more recent parts of the growth, where the vessel walls are still quite intact and the lumen appears entirely filled with typical tumor cells. It is not improbable that this thrombosis of the larger intrahepatic vessels, with subsequent rupture of the wall, may account in great part for the hemorrhagic character of the neoplasm.

The gray or cellular nodules of more recent development are composed of masses of large polyhedral cells with large

vesicular nuclei. These cells are packed together without definite arrangement. Here and there are found congested capillaries with endothelial lining intact with characteristic rows of cells surrounding them. The cells are loosely arranged apparently without definite connective tissue stroma. There are no hemorrhages in these nodules and but few areas of necrosis.

An apparent transition from hypertrophic liver cells into tumor cells is seen in various parts of the section, especially around the earlier hemorrhagic nodules before the cell characteristics have been destroyed by the compression of the blood spaces. The liver parenchyma around the tumor areas is usually compressed and the cells are atrophic and stain faintly. In many parts these liver cells resemble spindle cells and in places simulate a capsule. There is no definite capsule, however. The cells of the portion of the liver parenchyma which is free from compression are hypertrophied, stain intensely and show some mitotic figures. In many places the bile ducts appear increased in number but are normal in appearance. There is no cirrhosis. The liver capsule, however, is thickened and in places invaded by tumor cells. The nodule in the gall bladder is made up of dilated blood spaces lined with endothelium around which are arranged one or more rows of polyhedral cells possessing a clear cytoplasm and hyperchromatic nuclei, an arrangement which bears a striking resemblance to perithelioma.

Histogenesis. — The histological study of this particular case offers three distinct problems. First, the close relation of the tumor cells to the blood vessels, which is observed in certain portions of this tumor, their peculiar arrangement with reference to these channels and the many giant cells suggest an endothelial or perithelial origin, and would lead us to consider the term perithelioma for this atypical neoplasm, but the term perithelioma is applied to many varying types of neoplasms and our knowledge of true perithelial structures is very limited. The writer quotes briefly the

description of endoperithelioma given by Borst in considering the histogenesis of this disputed type of new growth:

"The peritheliomas which are classed in the literature as angiosarcoma (Waldeyer), perithelial angiosarcoma, perithelial sarcoma and perivascular endothelioma have the following structure in which the dilated capillaries, much less often the blood vessels, form the main part of the neoplasm. The cell growth takes place here, not as in the hemangioma simplex from the endothelial lining, but from the outer layer, so that all the many net-like vessels are surrounded by thick layers of flat, polygonal or even cuboidal or cylindrical cells. Toward the inner side of the cell coats one finds the endothelium which divides the perithelial cell masses from the contents of the blood vessels; therefore the cell mass rests directly on the endothelium.

"Either the epithelioid cells in the perivascular coats are placed next each other without interstitial tissue, or fine threads of fibrillar connective tissue and single spindle cells weave themselves like a delicate scaffolding through the perivascular cell complex. In the cell sheaths there is either a marked irregularity in the arrangement of the polygonal cells, or if there is a flattened type, the cells are placed in concentric layers on the endothelium or one finds not infrequently a radiating arrangement, where parallel slender columns of epithelial-like cells appear vertically placed on the axis of the vessels.

"Frequently the innermost cell layer, which is placed directly on the endothelium, assumes a cuboidal or even cylindrical form. When the connective tissue stroma of these tumors is poorly developed the new growth may consist entirely of tightly compressed blood vessels with thick coats. These are tumors of a plexiform type. If, however, the closely compressed cell layers fuse together, a sarcoma-like mass develops in the vessels. Often the walls of the older vessels thicken and in the surrounding endothelium a demonstrable quantity of fibrillar connective tissue is observed (the latter must be taken for a product of the endothelium of the vessels). Then the plexiform character is lost and an alveolar type of tumor is developed and we have the masses of tumor cells lying in the meshes of the network of blood vessels (Birch-Hirschfeld). By the marked increase of the connective framework between the vessels with the layers of perithelial cells an alveolar structure is formed and by it resemblance to carcinoma."

Roussy in a detailed study of perithelioma reaches the following conclusions:

"The perithelial structure may appear under so many different circumstances it does not seem advisable to form a distinct group of peritheliomas, except perhaps one based merely on morphological resemblance.

We recognize several types of tumors in which the characteristic perithelioma[illegible] appears:

"1. The peritheliomatous picture may be due to the normal histological structure of the organ which is the seat of the tumor. This is observed in tumors of the carotid gland, coccygeal gland, and adrenal, where it is only natural that tumors should reproduce the original typical structure of the organ.

"2. In certain tumors of connective tissue origin the perithelial feature is distinct but is capable of several interpretations. For example, in the central nervous system the vessels are covered with a lymphatic sheath which may become the seat of perivascular proliferation, producing perithelioma.

"3. In certain epithelial or connective tissue neoplasms, the perithelial type is merely a morphological feature resulting from the transformation of the tumor after necrosis or hemorrhage. Such for example in the case of connective tissue new growths are the neoplasms of the choroid, where, however, certain places may be found which demonstrate the true sarcomatous nature.

"In the case of epithelial tumors occurring in glandular organs one is dealing with an epithelioma modified by a degenerative process, hemorrhage or necrosis, where in the areas which remain active cells are arranged about the vessels that nourish them. The impartial proof of the value of this conception is found also in the fact that in all cases of this kind designated as perithelioma the co-existence of the process of necrosis and hemorrhage is mentioned.

"In conclusion, in a nomenclature already abundantly supplied with oncological terms, 'peritheliomas' occupy an illegitimate and entirely artificial place."

From this description we observe the manifold structures in which typical features of perithelioma may occur without regard to their derivation from, or even relation to, perithelial cells. In the writer's case there are many areas that show hemorrhage and necrosis, which are undoubtedly the chief factors in developing the peritheliomatous picture. It therefore does not appear justifiable to consider a perithelial origin for this atypical neoplasm.

The presence of large syncytial-like cells and giant cells in direct contact with the blood vessels suggests endothelioma or sarcoma. In view of the fact, however, that these peculiar large clear cells and giant cells are encountered in many types of tumors not ordinarily included under the heading of endothelioma or sarcoma and that almost any

rapidly growing tumor may assume the structure of an endoperithelioma, and since there are many other areas in this tumor which show the histological characters of diffuse typical carcinoma it does not appear permissible to classify this case with the sarcomas.

A second important question in the histological study of this case is the resemblance which certain areas bear to some forms of chorioma If we confine our study to those parts showing the disorderly arrangement of cells around blood masses, many large giant cells and syncytial cells, it cannot be denied that a faint resemblance to the structure of chorioma is observable; when, however, we study the smaller nodules with their rows of closely packed cells showing the rather orderly arrangement of the component parts this resemblance becomes extremely remote. The conclusion seems justified from the review of the literature that where true chorioma occurs in the liver its structure is readily recognized and there does not appear to be sufficient ground for identifying this tumor with heterotopic chorioma. It appears definitely impossible, too, to consider this case as a metastatic chorioma. We have seen that metastatic choriomas when they occur are highly typical, and in this case we have a patient who gives no history of a pregnancy and there was no primary tumor in the genital organs. Teratoid tumors of a choriomatous nature do not arise primarily in the liver.

A third significant feature observed in many areas of this tumor is the arrangement in rows of medium-sized tumor cells which appear to have many of the characteristics of liver cords. Occasionally these rows of cells are multiple and appear to have a normal relation to the capillaries. This histological structure is seen in the typical carcinoma derived from liver parenchyma, and also in the hemorrhagic forms of primary carcinoma. A comparison of this histological structure in my case with many areas described in the case reported by Ewing reveals the striking resemblance which

exists between these two types of neoplasm, and since his case was undoubtedly derived from liver cells this is strong evidence that the present case also originated from liver parenchyma, the variation in histological appearance being due to a difference in duration of the growth and not to origin.

The fact that tumors primary in the liver are derived from liver cells is without dispute. Undoubtedly the majority of typical carcinomas originate from liver parenchyma. Whether the hypertrophic parenchyma cells undergo transition into tumor cells is a point about which observers differ. Milne (1911) described in his three cases of liver carcinomas apparent transitions between hyperplastic liver cells and tumor cells. This transition has also been noted by v. Heukelom, Witwicky, Cloin, Wegelin, Polak-Daniels, Meyers, Muir, Yamagiwa, and others.

A study of the present case would seem to fully support the observations and deductions of the above observers since it presents direct evidence of the transformation of hyperplastic liver cells into tumor cells, with the formation of a variable but highly characteristic structure. This leads to the very interesting question whether these tumors develop from multiple foci or are the result of metastases from one original focus.

Beattie and Donaldson (1912) have described a case of primary carcinoma of the liver with multiple nodules which they believed to be derived from liver cells and they demonstrate a bile-like substance in the bile capillaries, also young tumor cells actually secreting bile. They did not observe trans tions from hyperplastic liver cells into tumor cells, and they hold the view that these multiple masses arise from one point and that the other foci are metastatic from this primary focus.

Oertel holds the opposite to be true and states very definitely "that these tumors are derived from multiple groups of liver cells, sometimes only involving a few cells in one lobule. These microscopic areas were best observed in those parts of the liver in which there was as yet no gross cancer formation and they demonstrated a direct change of atrophic degenerating liver cells while they were still in perfect continuity and still entered into the formation of the lobule."

In the writer's case the fact that no one mass larger than all the others could be demonstrated, and the presence of focal areas of transformation of liver cells, rather favors the view that this tumor developed from multiple foci, and the many nodules were not the result of metastases, but have a multiple origin from isolated focal areas of liver cells. These facts combined with a study of the literature furnish important information as to the histogenesis and manner of development of this neoplasm and lead the writer to consider this case as a primary hemorrhagic carcinoma of the liver, atypical in morphology, growing in certain areas as a perithelioma, multicentric in origin and derived from hypertrophic liver cells.

Relation to cirrhosis. — The relation which the hyperplasia of liver cells in these primary tumors bears to hyperplasia in cirrhosis is a point of considerable interest, and has led to many differing theories. A brief consideration of this subject seems indicated, as the case under discussion did not exhibit any cirrhosis.

Ribbert, Fischer, Lubarsch, and others have considered this hyperplasia as related to the regenerative changes observed in cirrhotic livers and believe there is a direct relationship between cirrhosis and carcinomatosis and that the cirrhosis precedes the development of the tumor. Thompson, Marckwald, and others hold that the cirrhosis is secondary to the tumor process. A third group of observers, including Hanot, Gilbert, and Frohmann deny any connection between cirrhosis and primary liver carcinoma. Neither of the three cases which the writer has had opportunity to observe were associated with cirrhosis, a fact which may serve to indicate that the cirrhotic process is not essential in this hyperplasia of liver cells. It is obvious that other factors than cirrhosis may be the cause of this regenerative change. Collateral hyperplasia occurring around tumor foci is a well known feature of tumor growth. This hyperplasia extends in the immediate neighborhood of the tumor focus and certainly cannot account for the diffuse hypertrophy observed in these cases.

Conclusions. — From the preceding study and a review of the literature, the writer offers the following deductions:

1. True hemangiosarcomas primary in the liver undoubtedly have been observed, but occur usually in early life; they are extremely rare and should not be confused with the atypical malignant tumors occurring in adult life. Endothelioma and perithelioma derived from the capillary structures of the liver are reported but without sufficient evidence, in the writer's opinion, to support the view that they are true endotheliomas. We have seen from the study of the classification of endoperitheliomas by Borst, and the review of this subject by Roussy, that the peritheliomatous picture is encountered in many varying types of tumors, not ordinarily classed as endothelioma or perithelioma and that any rapidly growing tumor may assume the structure of a perithelioma, in all probability due to their need for nourishment.

In the liver we have normally an organ in which the epithelial cells are in direct contact with the capillaries, which arrangement could very easily simulate perithelioma.

Therefore the ground on which Marx, Fischer, Kothny, and others base their interpretation of an endothelial or perithelial origin is quite inadequate and since structures similar to those which they depict appear in the present cases of primary carcinoma of the liver parenchyma the writer believes it probable that their tumors were also derived from liver parenchyma.

2. True chorioma primary in the liver has not been proved to exist. The resemblance to chorioma observed in certain cases proves on analysis to be very superficial, since these tumors do not show the typical Langhans cells and syncytium as do true choriomas even when heterotopic. The description of the case reported by Fischer, purporting to be true chorioma in the liver, leaves one in some doubt as to the origin of this particular tumor, and since structures closely resembling true chorioma have been observed in the cases of atypical hemorrhagic carcinoma studied we feel convinced that his case also in all probability took its origin from liver cells, and the choriomatous picture was produced,

as in the writer's case, by association with extensive hemorrhage and necrosis, with the production of huge giant cells, and large clear hypertrophic syncytial-like cells around blood masses.

3. Primary atypical carcinomas of the liver parenchyma show wide variations in histological structure, but usually exhibit in certain portions the definite arrangement of rows of cells simulating liver cords, with obvious transformation of hyperplastic liver cells into tumor cells. When we consider the histological structure of the liver it appears quite reasonable to suppose that rapidly growing tumors arising from liver parenchyma would be associated with hemorrhage and with the production of giant cells and that in the earlier areas the tumor cells would be arranged around dilated capillaries. This supposition is fully supported in the cases reported by Ewing, Cruickshank and Teacher, Goldzieher and Bokay and others and also in the present case. In all these cases the presence of hemorrhage and necrosis masked in certain parts the true nature of the neoplasm. The extent of this hemorrhage and necrosis in large measure determines many peculiarities noted by many observers in this atypical form of liver tumor.

An extensive histological study of many areas and a comparison of the histological structure of atypical hemorrhagic carcinoma with typical diffuse carcinoma of the liver will be necessary to demonstrate the true nature of these atypical neoplasms. Goldzieher and Bokay base their diagnosis on the typical liver cell characteristics encountered in these tumors which they believe proves the derivation from liver parenchyma.

Cruickshank and Teacher consider the apparent transformation of liver cells into tumor cells seen in certain areas as diagnostic. Ewing believes this transformation of liver cells into tumor cells associated with the arrangement of cells simulating liver cords as significant in demonstrating the true nature of these tumors.

4. The consideration of cirrhosis as an etiological factor in these cases does not appear to have sufficient justification.

In the three cases which the writer has had opportunity to study there was no cirrhosis, which rather favors the view that cirrhosis is not necessary in the development of this class of neoplasms and when present probably is of secondary importance.

Reviewing these conclusions: The evidence appears very strongly to favor an epithelial origin for these atypical tumors, and it seems advisable, therefore, to classify them with the primary diffuse carcinoma derived from liver parenchyma. The term "atypical hemorrhagic malignant hepatoma" seems most suitable.

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DESCRIPTION OF PLATES XI–XIII.

PLATE XI., FIG. 1. — Shows the liver with the tumor nodules on surface. The right lobe has been cut across to demonstrate the different types of tumor masses. The left lobe is uncut and shows the elevation of the tumor nodules above the surface of the liver. Gall bladder is distended and filled with stones

FIG. 2. — Small vessels dilated, surrounded by one or more rows of large cuboidal, in some places large polyhedral cells, which arrangement strongly suggests the picture of a perithelioma.

FIG. 3. — Tumor cells growing diffusely, showing many types of cells, a few giant cells, some almost like spindle cells. These areas were found in recently developed masses before the association with hemorrhage or necrosis.

PLATE XII., FIG. 4. — Early hemorrhagic area, with giant cells, large clear syncytial-like cells, and hypertrophic liver cells, in such close relation with tumor cells as to appear to be transformed into them.

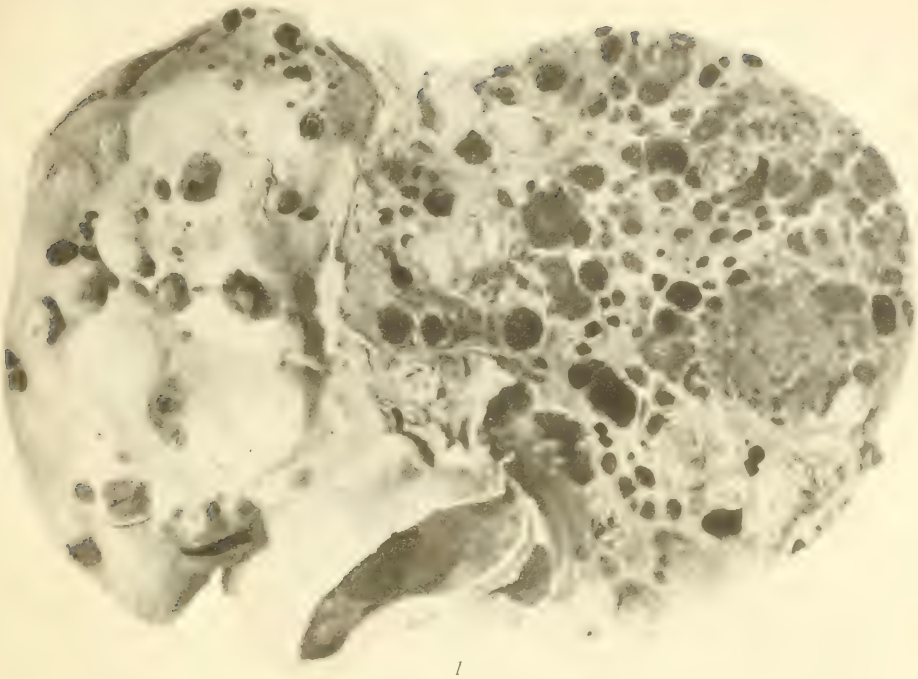
FIG. 5. — Masses of large clear cells with deep staining nuclei arranged in rows suggesting liver cords. There is no hemorrhage in these areas or beginning of necrosis.

FIG. 6. — In the wall of the hemorrhagic nodules one sees the blood coagulum surrounded by pseudo-Langhans cells and giant cells which penetrate to some degree into the blood mass. This arrangement has led many to consider this type of neoplasm as closely related to chorioma. However, even a superficial study will serve to demonstrate how remote this resemblance is in the majority of cases.

PLATE XIII., FIG. 7. — Blood vessels more or less completely surrounded by tumor cells. The irregular formation and varying types of cells also faintly suggest the picture of chorioma.

FIG. 8. — Distinct peritheliomatous arrangement seen in the case reported by Ewing.

FIG. 9. — Diffuse carcinoma showing arrangement in rows, of large hypertrophic cells, resembling true liver cords, with apparent direct transformation of liver cells into tumor cells. Also seen in the case reported by Ewing.



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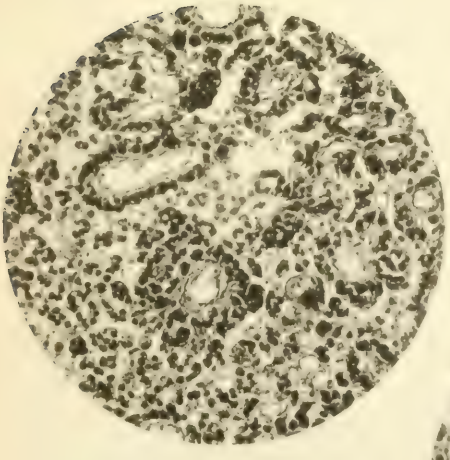

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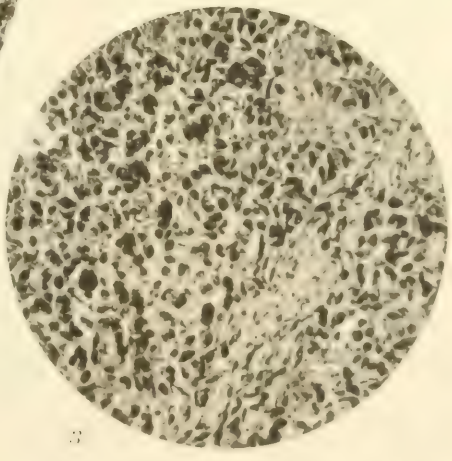

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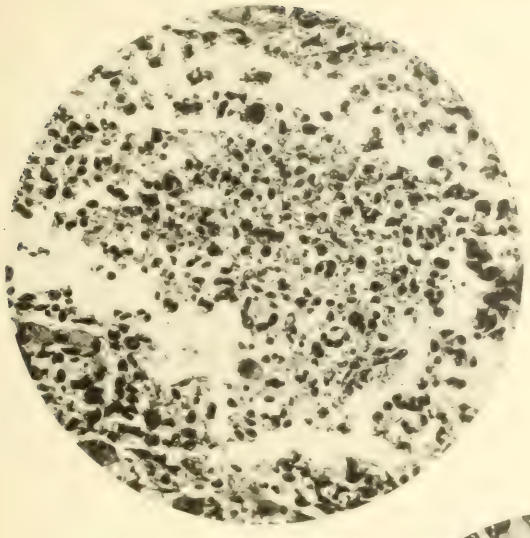

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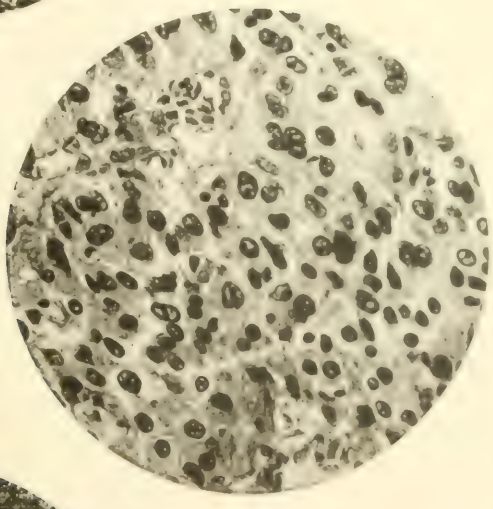

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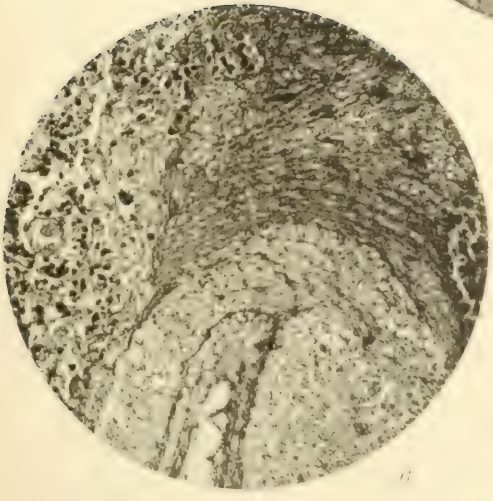

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TOXINS AND THE SIDE-CHAIN THEORY

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The toxins are distinguished from other poisonous agents by their property of stimulating the production of antibodies (antitoxins) when they are injected into animals. While, previous to the studies on cobra venom, nothing had been known regarding the mechanism of the action of toxins, that action had found an attempted explanation in the widely accepted side-chain theory of Ehrlich,[1] which considers not only the poisonous action of toxins, but also the mechanism of antitoxin production.

According to the side-chain theory, the toxin molecule is conceived to possess a chemical group by which it is capable of entering into firm chemical union, through a corresponding chemical group, with the sensitive tissue. The uniting group of the toxin molecule has been named the "haptophore" group and that of the vulnerable cell, "receptor." The union of toxin and cell having taken place—but not till then—the toxin is able to inflict its characteristic injury upon the cell, that injury being produced by a second chemical group, the so-called toxophore group. The tissue receptors that have united with the introduced toxin are assumed to have been thrown out of function in the cell economy and this elimination of the "receptors" is supposed to constitute in itself an injury that must be repaired. The injury caused by the mere union of the "haptophore" group with the cell receptors is different from that subsequently inflicted by the "toxophore" group, since the former injury must also be assumed, under the side-chain theory, to follow the injection of non-toxic antigenic substances, such as foreign blood sera or corpuscles. In repairing the defect, the cell is believed to produce a greater number of the "receptors" than were used up by the toxin. The excessive "receptors" are thrown off into the body fluids and constitute there the specific antibodies, or antitoxins. In explaining this excessive production of the "receptors," Ehrlich has cited the analogous excessive production of tissue in the repair of the

1. Proc. Roy. Soc., 1900, 66, p. 424.

loss of substance following injuries. This parallel clearly expresses the conception that the union of haptophore group and cell receptor results in injury to the cell, for there is in nature no known instance of a loss of tissue not accompanied with injury that is followed by an overproduction of that tissue.

It should be remarked here in passing that the laws governing the response of the cells to antigenic stimulation are quite different from those controlling the response of tissues to the effect of injurious agents; that is, to irritants. It is a fact of common knowledge that if a tissue be exposed at intervals to the influence of an irritant, that tissue becomes increasingly resistant to the irritant and the cell multiplication, which is the index of reaction on the part of the tissue, becomes less and less. In their discussion of this point, von Dungern and Werner[2] write:

> If a definite irritation be repeated at intervals of sufficient length, so that in the interim the cells may return to the resting state, an important phenomenon will be observed. The reaction to the irritant becomes less and less, until finally the same degree of irritation that at first set up a vigorous growth is no longer able to produce any increase in proliferation. If an irritant be applied continuously or at very short intervals, the end result is different according to the intensity of the irritation. If the latter be mild, an increased power of resistance against the growth-producing irritant takes place, so that the proliferation not only does not progress, but at last actually diminishes. If, on the other hand, the intensity of the irritant is great, the recovery of the cells from the injury is prevented, and, as a result of a cumulative action of the irritant, an increase of growth takes place up to a definite maximum, to be followed, upon further irritation, by the death of the cell.

On the other hand, tissues that are exposed at intervals to antigenic stimulation exhibit, at first, more and more sensitiveness to that stimulation, as is shown by the increased production of the specific antibodies, up to a certain height, after which, instead of a power of resistance, or hyposensitiveness, developing against the antigenic stimulation, the response may continue unabated for an indefinite period, subject only to the condition of the general health, which, it must be remembered, may be affected by the injections of the antigen.

The effect of repeated injections carried out over an extended period has been exactly studied by Elser and Huntoon, to whom I am greatly indebted for permission to refer to some of those studies in advance of their final publication: Four rabbits, 441, 234, 286, and 285, received daily intravenous injections of the same small amount of

2. Das Wesen der bösartigen Geschwülste., 1907.

meningococci over periods of 13 months, 12 months, 8 months, and 30 months, respectively (the last animal receiving 1091 injections). In all of these animals, the rate of antibody production increased rapidly, at first, up to a maximum, after which, with the exception of certain fluctuations of greater or less extent, which often could be referred to conditions affecting the general health of the animal, such as pregnancy and infection, the rate remained relatively high. In no instance was a decline observed in the rate of antibody production that could be referred to a repetition of the injections themselves. Two other rabbits that received similar injections of typhoid bacilli over a period of three months responded in precisely the same way.

Two rabbits that I have treated in the same manner with sheep blood corpuscles (0.1 c.c. of blood daily) yielded a hemolytic serum of a strength of 1 : 40,000, which was maintained practically unchanged for a period of several weeks.

It is seen that the reaction of the tissues to antigenic stimulation is different from that of tissues to injurious irritation. This antithesis, which hitherto has been overlooked, cannot be compromised; it prevents the application of the principle of Weigert to the explanation of the mechanism of antibody production. The reaction of the tissues to antigenic stimulation finds a more plausible physiologic analogy in the response of digestive glands to specific chemical stimulation offered by foodstuffs than in the response of tissues to injurious influences.

The toxins bear a long-recognized, striking resemblance to the organic ferments. This resemblance, however, has not led to any general belief in the fermentative nature of the toxins. On the contrary, the only writer on this question in recent times, von Liebermann,[3] emphatically denied that the toxins are ferments. Moreover, von Liebermann made the noteworthy remark that the establishing of the fermentative nature of the toxins would render the side-chain theory untenable. Von Liebermann based his denial of the fermentative nature of the toxins upon the results of his experiments with the agglutinating property of ricin and abrin. Those results are as follows: (1) The agglutinating principle of these preparations is quickly absorbed in a definite quantitative proportion by the blood corpuscles, that is, these "toxic" bodies are "used up" in the reaction, whereas the ferments, theoretically, are not used up. (2) The ricin agglutinin is

3. Deutsch. med. Wchnschr., 1905, 31, p. 1301.

not appreciably affected by hydrocyanic acid, which is a "violent enzyme poison." (3) The agglutinating property of ricin is not destroyed by exposure for half an hour to a temperature of from 70 to 80 C.; that is, this "toxic" body is not thermolabile as are the ferments. Von Liebermann recognized that the incompleteness of our knowledge about ferments could be urged against drawing any deductions regarding the fermentative nature of toxins, but believed nevertheless that that knowledge was sufficient to support the evidence on which he based his denial. However, it is just at that point that his whole argument has fallen; for since he published these views a ferment has been found that is absorbed by the substratum upon which it acts (appearing thus to have been used up), that is not appreciably affected, as we shall show, by hydrocyanic acid, and that is not affected by exposure for half an hour to a temperature of over 70 C. The ferment referred to is the lipase of cobra venom, which is also the hemotoxin of the venom.

The absorption of the lipase of cobra venom by lecithin was first demonstrated by Kyes and amply confirmed by von Dungern and Coca, and Manwaring. Von Dungern and Coca showed that the absorbed lipase was not actually used up, but could be demonstrated in active condition in the digestion products of the lecithin, and later Manwaring was able to isolate the lipase again from those digestion products.

Therefore, the fact that a toxin is absorbed by the vulnerable tissue does not show that the toxin is used up in the process of its activity; hence, its ferment nature cannot be disputed on this ground.

We have investigated the influence of hydrocyanic acid upon the lipase of cobra venom and have found that the lipolytic action of the ferment is not interfered with by this acid.

Six and one-half grams of potassium cyanid (Kahlbaum, 96-98 percent pure) were mixed with 47 c.c. of strong HCl (10 c.c.-2.9 c.c. normal), and the final volume of the mixture was then brought up to 50 c.c. by the addition of distilled water. The resulting fluid thus contained about 5.2 percent of hydrocyanic acid and 15 percent of potassium chlorid. The reaction of the fluid was very slightly alkaline to litmus, and twenty drops of it caused no hemolysis of 1 c.c. of a 5 percent suspension of ox blood corpuscles.

Four dilutions of cobra venom (for which I am under obligation to Prof. A. Calmette, director of the Pasteur Institute in Lille), were prepared containing, respectively, 1 in 10,000, 1 in 100,000, 1 in 1,000,000, and 1 in 10,000,000 of the venom. Three series of twelve tubes each were arranged, each series containing 1 c.c., 0.5 c.c., and 0.2 c.c. of the four venom dilutions, and each tube containing 0.1 c.c. of a 0.2 percent emulsion of lecithin, which by itself was not hemolytic in a quantity of 0.4 c.c.

To each tube of Series A was added 1 c.c. of the hydrocyanic acid solution (von Liebermann used 0.2 c.c. of a 72 percent solution) and to each tube of Series B was added an equal volume of a 15 percent solution of potassium chlorid. No addition was made to the third series, C.

After these three series had stood for twenty-four hours at room temperature, 1 c.c. of a 5 percent suspension of ox blood was added to each tube and the hemolysis resulting at room temperature was observed. In the first three tubes of Series C, hemolysis was complete in a few minutes, whereas in the corresponding tubes of Series A and B complete solution occurred much later (from one to three hours). At the end of a further twenty-four hours, the tube of Series C containing 1 c.c. of the fourth dilution showed complete hemolysis; that containing 0.5 c.c. of the fourth dilution showed strong hemolysis; and that containing 0.2 c.c. of the fourth dilution showed a trace of hemolysis. In Series A, the corresponding tubes showed complete hemolysis, slight hemolysis, and a trace of hemolysis. In Series B, the corresponding tubes showed strong hemolysis, no hemolysis, and no hemolysis. It is seen that not even during twenty-four hours contact was the hydrocyanic acid able appreciably to interfere with the lipolytic activity of the venom.

The relative resistance of the cobra venom lipase to heat is already known. Heating a 1 percent solution of the venom for three quarters of an hour at 72 C. does not noticeably affect its lipolytic activity, which is undiminished even by short boiling.

All of the arguments brought by von Liebermann against the theory of the fermentative nature of the toxins are thus seen to have lost their force through the subsequent studies on the lipase of cobra venom. Moreover, these same studies have demonstrated that one toxin (the hemotoxin of the venom) is actually a ferment; in other words, the hemotoxin has been shown to be identical with the lipase of the venom.

The hemotoxin of cobra venom is that constituent of the venom which destroys red blood corpuscles. Not all species of red blood corpuscles are susceptible to the direct action of the hemotoxin; those of the guinea-pig and man, for example, being very susceptible, and those of the ox and the sheep being non-susceptible. The red blood corpuscles that are invulnerable to the direct action of the hemotoxin can be made vulnerable with the co-operation of certain "activators," such as normal serum and lecithin. The mechanism of the "activation" is not the same with these two agents. The destruction of the corpuscles is brought about in the two cases through the action of two different constituents of the venom. This is shown by the fact that, on the one hand, treatment of the normal serum in the cold with the invulnerable corpuscles greatly diminishes the activating power of the serum, and that, on the other hand, heating the venom solution in an

acid medium causes it to lose its power of destroying the invulnerable corpuscles when it is mixed with normal serum, altho it is still able to do so in co-operation with lecithin.

The present discussion concerns itself only with the venom constituent that attacks the naturally invulnerable corpuscles with the aid of lecithin. The researches of Kyes, Kyes and Sachs, von Dungern and Coca, and Manwaring have shown that the venom-lecithin hemolysis is brought about by the fermentative action of the venom on the lecithin whereby the latter, a non-hemolytic substance, is split into two parts both of which are strongly hemolytic. These two parts are oleic acid and the lecithin rest, which is lecithin in which one of the two fatty acid molecules is missing. Because of the demonstrated fact that this mono-fatty acid lecithin rest contains no oleic acid, it has been designated "desoleolecithin." The properties by which this substance is recognized are, its solubility in alcohol and water, its insolubility in ether, and its great hemolytic power.

It has been assumed that the mechanism of the direct hemolytic action of the cobra hemotoxin in destroying naturally vulnerable corpuscles is the same as that of its indirect hemolytic action in co-operation with lecithin. The immediate hemolytic agents in the former case would thus be the split products of the lecithin that is normally present in the red blood corpuscles in a quantity sufficient to supply a completely hemolytic amount of desoleolecithin and oleic acid. In accordance with this assumption, it was conceivable that desoleolecithin could be demonstrated in the fluid resulting from the direct hemolytic action of the venom on naturally vulnerable corpuscles. The following experiments will show that such demonstration is possible.

A preliminary experiment was carried out in order to find out what part of a definite quantity of desoleolecithin that has been mixed with blood corpuscles can be recovered with the method at our disposal.

One hundred cubic centimeters of ox blood were well washed with physiologic salt solution and the corpuscular sediment was mixed with 0.2 gm. of desoleolecithin (two complete hemolytic doses) which had been dissolved in salt solution. (The minimal completely hemolytic quantity of this preparation for 1 c.c. of 5 percent ox blood was 1/20,000 gm.) After the resulting hemolysis was complete, 200 c.c. of distilled water and 100 c.c. of 95 percent alcohol were added and the mixture was boiled for a few minutes. The fluid, which will be referred to as the "first extract," was separated from the coagulated proteins by filtration. The coagulated proteins were mixed with 400 c.c. of 95 percent alcohol and allowed to stand over night at room temperature.

The first extract was evaporated at first by boiling and finally with the use of an electric fan, and the residue was extracted with ether. The ether extract

was discarded and the residue was again extracted with warm absolute alcohol. The alcoholic extract was evaporated with the use of the electric fan, and the residue was taken up in 20 c.c. of physiologic salt solution. One cubic centimeter of a 5 percent suspension of washed ox blood was completely hemolysed in a few minutes by 0.4 c.c. of this solution and slightly dissolved by 0.2 c.c. of the solution. The entire 20 c.c. therefore contained enough of the hemolysin to dissolve completely 2.5 c.c. of undiluted ox blood.

The second alcoholic extract of the coagulated proteins of the ox blood was obtained by filtration after the mixture of the proteins and after the alcohol had stood over night at room temperature. The filtrate was evaporated to dryness with the use of the electric fan, and the residue thus obtained was extracted with ether, the ethereal extract being discarded. The residue was then extracted with warm absolute alcohol and the alcoholic extract, separated by centrifugation, was evaporated to dryness and the resulting residue taken up in 10 c.c. of warm physiologic salt solution. This solution hemolysed 1 c.c. of 5 percent ox blood in a minimal quantity of 0.02 c.c., and the entire 10 c.c. contained, therefore, enough of the hemolysin to dissolve completely 12.5 c.c. of undiluted ox blood.

The hemolysin obtained by the procedure just described was identified as desoleolecithin, first, by its solubility in alcohol and water, and its insolubility in ether, which distinguishes it from the hemolytic substances in the extracts of normal tissue; secondly, by the rapidity of its hemolytic action, the minimal dose producing complete hemolysis within a few minutes; and finally, by reason of the failure of this method of extraction, in several experiments, to demonstrate any such hemolysin in corpuscles that had not been dissolved either with cobra venom or desoleolecithin. The experiment therefore demonstrates that even if a quantity of desoleolecithin capable of completely dissolving 200 c.c. of undiluted ox blood be mixed with only 100 c.c. of such blood, it is possible, with the method of extraction used, to recover from the hemolyzed corpuscles only about 7.5 percent of the desoleolecithin taken. By far the greater part of the hemolysin was combined with the corpuscular substance in such a way that the method of extraction employed failed to separate it.

The same method of extraction was applied to guinea-pig blood corpuscles that had been hemolyzed either with distilled water or with native cobra venom. In the former instance no hemolytic substances at all were obtained, but in the latter instance a quantity of hemolysin having the properties of desoleolecithin previously mentioned was obtained that was equivalent to about 2.5 percent of the calculated minimal amount necessary completely to dissolve the corpuscles used

For this experiment 400 c.c. of defibrinated guinea-pig blood were well washed with physiologic salt solution and the corpuscular sediment was divided into two equal portions. To one portion was added 0.1 gm. of cobra

venom dissolved in 5 c.c. of physiologic salt solution. After three quarters of an hour at room temperature, microscopic examination showed that the corpuscular forms had completely disappeared and that the thick fluid contained masses of hemoglobin crystals. At the end of two hours, 100 c.c. of distilled water were added to each portion of blood and both portions were treated according to the method of extraction previously described. Thirty cubic centimeters of the final salt solution extract were obtained from each portion. That derived from the blood that had been dissolved with distilled water lacked completely any hemolytic power; that derived from the portion of blood that had been dissolved with cobra venom was completely hemolytic in a minimal quantity of 0.3 c.c.

The entire amount of the extract thus contained enough of the hemolysin to dissolve 100 c.c. of 5 percent guinea-pig blood, or 5 c.c. of undiluted blood, which is 2.5 percent of the quantity in each portion of blood used for the experiment.

A similar experiment, the notes of which have been lost, led to the same result; a considerable quantity of the hemolysin possessing the properties of desoleolecithin was again extracted from guinea-pig corpuscles that had been dissolved by the direct action of cobra venom.

The same method of extraction was applied also to ox blood corpuscles that had been dissolved by the combined action of anti-ox corpuscle amboceptor serum and normal guinea-pig serum, with negative result, the method of extraction failing to discover the production in the dissolved corpuscles of any hemolytic substance possessing the properties of desoleolecithin.

The sediment of 200 c.c. of well-washed ox blood was mixed with 25 c.c. of the serum of a rabbit that had received injections of ox blood, and to this mixture were then added 200 c.c. of normal guinea-pig serum. Two hours later, complete hemolysis having taken place in thirty-five minutes, the mixture was treated with the alcohol and ether extraction method. The final residue was taken up in 3 c.c. of salt solution, and this solution was then found to be entirely lacking in hemolytic activity when mixed with 1 c.c. of 5 percent ox blood.

The results of the experiments just described leave no room for doubt as to the mechanism of the direct destructive action of the cobra hemotoxin. It is clear that this hemolytic action is brought about by the lipolytic power of that constituent of the venom whereby the normally contained lecithin, and no doubt other fatty substances as well, are split into products that are the ultimate cell-destroying substances. It follows, therefore, that the cell destruction in this instance cannot be due to any assumed chemical group (toxophore group) in the toxin molecule.

Furthermore, if any of the toxin does attach itself to the cell proper according to the conception of the side-chain theory (through a haptophore group), as has been shown by von Dungern and Coca[4] to occur when naturally immune corpuscles (ox corpuscles) are brought into contact with a solution of cobra venom, the cell is not injured thereby. It is only when the toxin reaches the lecithin, becomes dissolved in it, and splits it into desoleolecithin and oleic acid, both of which are highly injurious to the cell, or when it reaches some other fatty substance and splits it into glycerin and some lower fatty acid, both of which are cell poisons—only then does the injurious influence of the hemotoxin reveal itself.

The two important results that have come out of the investigations of the hemotoxin of cobra venom are: First, the demonstration of the fermentative nature of a toxin, and second, the demonstrated failure of the side-chain theory in the first real test to which it has been put as an explanation of the mechanism of toxin action. In view of the first of these results, it seems safe to assume that all toxins may be ferments. The sources of the ferments and those of the toxins are the same. Ferments as well as toxins are known to exist in glandular secretions of animals and in the products of the growth of bacteria and in the higher forms of plant life. We have already recalled the long-recognized similarity between the general properties of ferments and those of toxins.

Another result of the investigations of the hemotoxin of cobra venom is the explanation of the natural resistance of the invulnerable corpuscles (ox and sheep) to the direct action of the hemotoxin. This natural resistance, or immunity, has been found to depend upon a physical condition of the cell substance that prevents the hemotoxin from penetrating to the lipoids of the corpuscles. This was shown by the discovery of Goebel[5] that the mere suspending of the naturally immune corpuscles in a chemically inert solution of sugar suffices so to alter the physical condition of the corpuscular substance that the hemotoxin can then enter the cells, reach the lipoids, and cause hemolysis. Other substances, also, such as soap and oleic acid, have been found to produce a physical alteration of the corpuscles that is similar in effect to that produced by the sugar.

As the discovery of the nature and manner of action of the hemotoxin of cobra venom has revealed the probable nature of the other

4. München. med. Wchnschr., 1907, 47, p. 2317.
5. Compt. rend. de Soc. de biol., 1905, 58, p. 422.

toxins, so also the explanation of the natural immunity of the invulnerable corpuscles to the direct action of the venom hemotoxin may provide a clue to the mechanism of some of the other known instances of natural immunity to toxins.

It is evident that the explanation offered by Ehrlich[1] for natural immunity cannot be brought into harmony with the demonstrated mechanism of the natural immunity of blood corpuscles to the direct action of the hemotoxin of cobra venom. Ehrlich's explanation was that the cells of the naturally immune animal do not possess receptors capable of entering into chemical union with the haptophore group of the respective toxin molecule. This explanation is incompatible with the demonstration of Goebel that the natural immunity of invulnerable corpuscles to the direct action of cobra hemotoxin is dependent upon a physical condition of the corpuscular substance.

Of the numerous cases of natural immunity to toxins that have been studied, only few are known (such as that of the scorpion to its own venom and that of the hedge-hog to the viper's venom) which could be referred to any antitoxic power of the blood. Indeed, some of the naturally immune animals are not capable of producing antitoxic substances, even after having received large injections of the toxin.

There are a few cases known in which a relative natural immunity is present so long as the body temperature of the animal remains below a certain point. The frog, in winter, and the hibernating bat and marmot possess such a relative resistance to tetanus toxin. At the higher "summer" temperature, all these animals become susceptible.

The remarkable natural immunity of the fowl against tetanus toxin remains an inexplicable phenomenon. The blood of the fowl is entirely lacking in antitoxic property; the immunity is therefore not humoral. Under ordinary condition of health, large subcutaneous or intraperitoneal injections of the toxin are borne by fowl without symptoms; but, if the injections are made into the brain or after the fowl have become weakened by cold, the toxin is able to produce its characteristic effect. It is conceivable that in this case, as well as in a number of other instances in which the immunity is demonstrably not humoral, the physical condition of some protective tissue or of the sensitive cells themselves plays a determining role.

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A RAPID AND EFFICIENT METHOD OF PRODUCING HEMOLYTIC AMBOCEPTOR AGAINST SHEEP CORPUSCLES

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From the time when the principle of complement-deviation was applied to the serum diagnosis of disease, as in syphilis, gonococcic infection, glanders, and malignant tumor, the possession of a "good" hemolytic immune-serum, usually prepared against sheep corpuscles, became a necessity to the clinical laboratory. By a "good" hemolytic serum is meant one that possesses a sufficiently high hemolytic strength, which must remain permanently constant.

The usual method of producing hemolytic sera has been to inject relatively large and increasing quantities (5-20 c.c., in some laboratories even as much as 50 or 75 c.c.) of the washed corpuscles at intervals varying from three days to one week, until four or five injections have been given, the bleeding of the animals taking place about one week after the last injection. The results obtained by this procedure have been often unsatisfactory, it being almost exceptional for the hemolytic serum thus produced to possess both of the requirements mentioned.

Following the publication by Fornet and Muller[1] of experiments showing that precipitating sera of high potency can be produced by injecting the antigen in increasing amounts on three successive days, this rapid method of immunization was applied by Bonhoff and Tsuzuki,[2] without success, to the production of hemolytic sera against the corpuscles of the sheep, the horse, man, and the pig. The titer of the resulting sera was uniformly low, 1 : 10.

More recently Gay and Fitzgerald[3] repeated these experiments of Bonhoff and Tsuzuki, using only sheep blood corpuscles and injecting quantities of 1 or 2 c.c. intravenously. According to Gay and Fitzgerald, such injections, administered on three successive days, were fol-

1. Ztschr. f. biol. Technik u. Methodik, 1[illegible], 1, p. [illegible]
2. Ztschr. f. Immunitätsforschung, 1[illegible], 4, p. 1[illegible]
3. University of California Publications in Pathology, 191[illegible], [illegible], p. [illegible]

lowed by the development of an unusually strong hemolytic property in each of the eight animals used for the experiment.

For a number of years, the writer has studied the production of hemolytic sera in rabbits and has been able, on the basis of many observations, to formulate some generalizations that are of practical value to routine serology.

1. The first of these generalizations is the well-known fact that the individual rabbits differ greatly in their response to the injection of the foreign corpuscles. These individual differences, which are both quantitative and qualitative, are illustrated in the following experiment:

Six normal rabbits received, on June 16, 2 c.c. each of washed sheep corpuscles, intravenously injected, and on June 21 a second intraperitoneal injection of 10 c.c. each. On June 30, the sera of the rabbits were examined as to their hemolytic activity. In these tests, as in all the subsequent tests of hemolytic strength referred to in this communication, the different reagents were combined in one-tenth of the usual quantities, i. e., the different hemolytic sera were first diluted 1:10 and then mixed in descending amounts with 0.1 c.c. of a 5 percent suspension of washed sheep corpuscles (based on the volume of full blood taken and representing about 2.5 percent of corpuscular sediment) and 0.1 c.c. of a 1:10 dilution of fresh guinea-pig serum. The smallest quantity of the diluted hemolytic serum that caused complete hemolysis of the sheep corpuscles was noted as indicating the relative hemolytic strength of the serum.

The results of the test were:

In Rabbits 11, 12, 15, and 16	0.0002
In Rabbit 10	0.0004
In Rabbit 9	0.0008

Five weeks later, the sera that had been obtained from Rabbits 11, 12, 15, and 16 on June 30 and preserved by the addition of 0.25 percent of carbolic acid, were tested again. The results of this test with Sera 11, 15, and 16 were identical with those obtained at the first examination; whereas the titer of Serum 12 had fallen to about 0.0008, i. e., to about one-fourth of its original strength.

It is seen that the same previous treatment in the six rabbits resulted in a high hemolytic potency in only four of the animals, and that, of these four, only three furnished hemolysins that were entirely stable.

2. It has been found that the quality of the hemolysins produced by means of many injections is often different (as judged by the criteria of stability and anti-complementary property) from those obtained after few injections.

This qualitative difference was clearly seen when the immunization of Rabbits 11, 15, and 16 was resumed, as follows:

From August 9 until August 16, inclusive, daily intravenous injections of 0.2 c.c. of washed sheep corpuscles; August 25, 27, and 29 similar injections; September 23, 25, and 26 similar injections of 1.0 c.c. On October 2 — six days after the last injection — the sera of the three animals were examined; they were all found to possess the same hemolytic potency, namely, 0.0001. It was observed that when the larger quantities of the hemolytic sera (0.002 c.c. or more) were combined with the unit of sheep corpuscles, if complement were immediately added, hemolysis failed to occur; whereas, if the addition of complement was deferred for about fifteen minutes, solution of the corpuscles then followed. This phenomenon has never been noticed in testing hemolytic sera that had been obtained after few injections of the corpuscles. One-quarter of 1 percent of carbolic acid was mixed with the sera and after three weeks, during which the sera had been kept in the ice-box, they were again examined; it was found that all three of them had lost over 90 percent of their original strength. The titers were: Serum 11, 0.0015, Serum 15, 0.0014, and Serum 16, 0.0012. There was no bacterial growth in any of the sera. After another interval of one month, Sera 15 and 16 were found to have exactly the same hemolytic power as at the last examination. Serum 11 had been lost by accident.

These experiences, confirmed in a number of other rabbits that had had similar treatment, show that after many injections made over a relatively long period of time the resulting hemolytic property of the serum is often in large part—over 90 percent—unstable; whereas after few injections—two in the present instance—the same animals usually yield sera the hemolytic potency of which remains entirely constant; they show, furthermore, that hemolytic sera obtained after many injections of the corpuscles possess certain anticomplementary properties, which are not found in such sera obtained after few injections.

3. It was found that the maximal degree of immunity can be effected by injecting relatively small quantities of the blood corpuscles.

The most powerful hemolytic sera that we have examined were prepared by giving daily intravenous injections of as little as 0.1 c.c. of washed sheep corpuscles for a period of many weeks. Such sera have been hemolytic in quantities of 0.00005 and 0.000025 c.c. A hemolytic serum of equally high strength has been obtained by Dr L'Esperance with the same procedure.

We have already demonstrated (under 2) that the important quality of stability of hemolytic strength combined with high pontency can be secured only by the administration of few injections, and, under this condition, as the following experiment shows, the maximal effect

cannot be produced with so small an amount as 0.1 c.c.* One cubic centimeter, however, was found to be sufficient.

Six normal rabbits that had received two intravenous injections of 0.1 c.c. of washed sheep corpuscles at intervals of four to six days yielded hemolytic sera of an average strength of 0.001. Four other normal rabbits that had received two similar injections of 1.0 c.c. each at an interval of five days yielded hemolytic sera of an average strength four and one-half times as great—0.00022. Nine additional normal rabbits receiving primary injections of 1 or 2 c.c. and second injections of 5 or 10 c.c. yielded sera possessing an average potency of 0.00025.

From the results of this experiment it is seen that no greater hemolytic activity is obtained by injecting 5 or 10 c.c. than by injecting 1 c.c. of the corpuscles.

4. It has been found that the optimal time for making the second injection of the sheep corpuscles is not earlier than at the end of an interval of three days after the first injection; that is, on the fourth day of the treatment.

The considerable advantage obtained by making the second injection after an interval of not less than three days is graphically shown in the accompanying table. The height of the black columns represents the average relative concentration of the hemolytic substances in the blood after the different immunizing procedures.

It is seen that the low average hemolytic power resulting from a single injection is considerably increased by further injections undertaken on the second and third days—Columns 2 and 3. This increase, however, is much greater if the second injection is deferred till the fifth day—Column 4.

5. The height of the hemolysin production following the first injection of sheep corpuscles is reached after an interval of seven days, and following the second injection the new height is reached after a further interval of not more than five days.

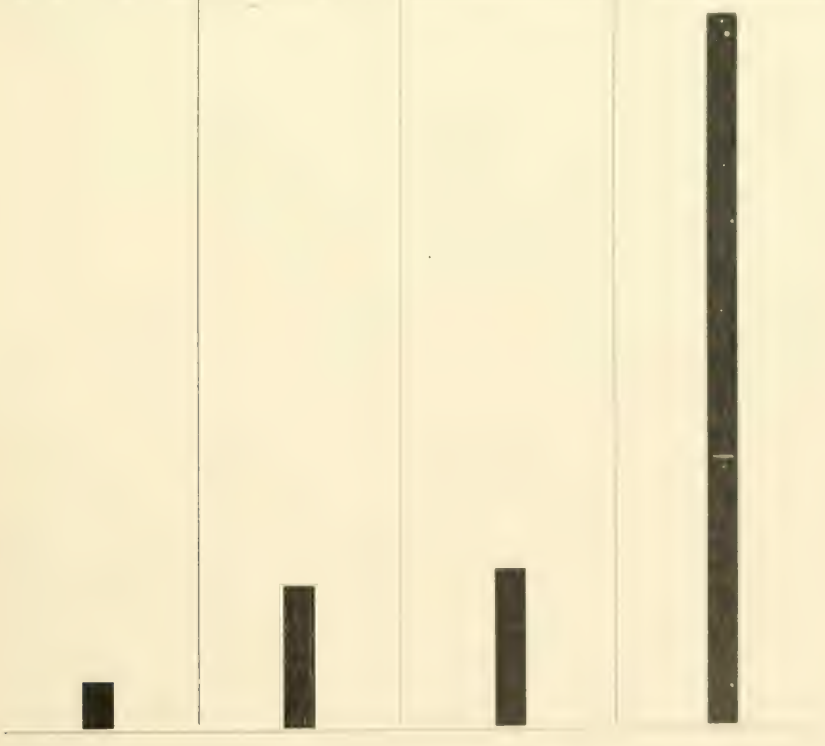
In the accompanying chart is shown the course of the hemolysin production in six rabbits that received two intravenous injections each

* This statement applies only when more than a single injection are given, as the following experience shows: Ten normal rabbits received 0.1 c.c. each of washed sheep blood corpuscles, intravenously administered, and one week later these animals yielded hemolytic sera of an average strength of 0.004. Three other normal rabbits that had received similar injections of 2.0 c.c., that is, twenty times as much as the former series, yielded an average hemolytic power of only 0.0065. This average would, no doubt, have been higher if a larger number of animals had been used; however, it is safe to assume that even then the average hemolytic power would not have been greater than that obtained in the series in which 0.1 c.c. of the corpuscles was injected.

TABLE 1

THE ADVANTAGE OF A FOUR-DAY INTERVAL BETWEEN THE INJECTIONS OVER A TWO-DAY INTERVAL AND THE SO-CALLED "RAPID METHOD" — COLUMN 3 — IN THE IMMUNIZATION OF RABBITS AGAINST SHEEP CORPUSCLES

AVERAGE TITER OF			
Thirteen Rabbits Receiving a Single Injection	Three Rabbits Receiving Two Injections, the Second Injection being Given on the Third Day	Four Rabbits Receiving Injections on Three Successive Days	Four Rabbits Receiving Two Injections, the Second Injection being Given on the Fifth Day
0.005	0.0012	0.001	0.00022



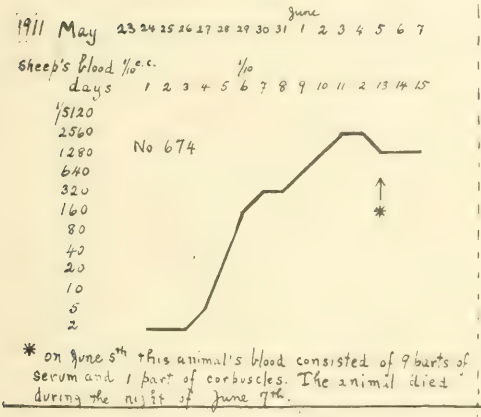
of 0.1 c.c. of washed sheep corpuscles at intervals of five, six, and seven days.

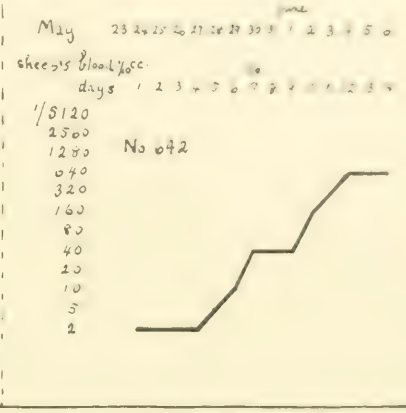
Following the first injection, the hemolysin content of the blood began to rise in all the animals after an interval of four or five days and increased daily up to the seventh day. Then, with one exception —Rabbit 684—the hemolytic strength remained unchanged for two days (Rabbits 674 and 643) or for three days (Rabbits 647, 642, and 644). This level marks the height of the response to the first injection. The effect of the second injection began, in some instances (Rabbits 674 and 642), to be apparent after three days, and in no animal was there any increase in the hemolysin content of the blood after the fifth day. The exceptional course of the curve in Rabbit 684, in which there was no pause in the increase of the rate of hemolysin production up to the final maximum, is due perhaps to two factors: first, the response to the second injection may have begun earlier than in the other rabbits; and secondly, the hemolysin content of the blood in this animal on the seventh day after the first injection was so low—1:40—that a relatively small increase could be easily detected.

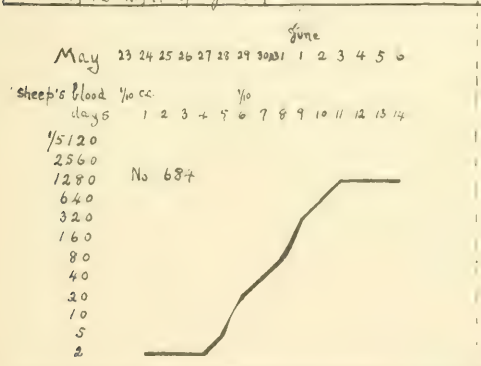
The curve of hemolysin production as determined in this study differs in two respects from that of precipitin production as found by von Dungern. (a) In one instance, his rabbit "A," the height of the precipitin production was not reached until the eighth day after the first injection of the Majaplasma, and in the same animal the height of precipitin production following the second injection was not reached until the sixth day. (b) After reaching its height on the seventh or eighth day, the curve of precipitin production rapidly descends to a point at which it remains for a time stationary, whereas the curve of hemolysin production remains stationary from the day on which the height is reached.

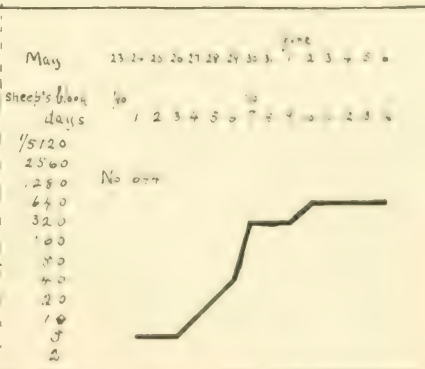
The results of the preceding study may be applied as follows to the practical production in rabbits of a hemolytic serum against sheep corpuscles.

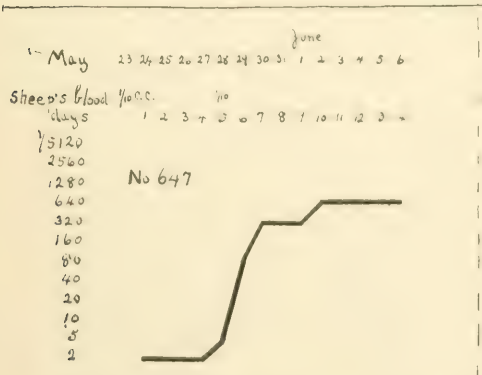
Two intravenous injections of 1, or at most 2 c.c. each of washed sheep corpuscles should be given at an interval of not less than four days to three rabbits. At the end of five days after the second injection, at least one of the rabbits will almost always yield a strongly hemolytic serum the hemolytic potency of which will be stable. If it is desired not to kill the animals, almost as much blood usually can be obtained by bleeding from the ear vein on two successive days (say











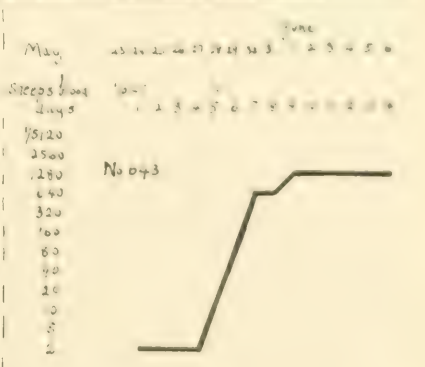


Chart 1.—The curve of hemolysin production in rabbits receiving two intravenous injections each of 0.1 c.c. of washed sheep corpuscles.

the sixth and seventh days after the second injection) as by bleeding to death. The potency of the serum obtained from the second bleeding is as high as that obtained from the first bleeding. The rabbits should not be used again for the production of amboceptor against sheep corpuscles.

In a single experiment in immunization with ox corpuscles, we have obtained results that are comparable with those obtained with sheep corpuscles.

Three normal rabbits received, at an interval of five days, two intravenous injections each of 1 c.c. of washed ox corpuscles. On the sixth day following the second injection, the hemolytic strength of the sera of the three rabbits was respectively 1 : 2500, 1 : 2500, and 1 : 320. After two months, during which the sera had been preserved in the ice-box with the addition of 0.25 percent of carbolic acid, the hemolytic strength of the sera was found to be unchanged.

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A NEW MEMBER OF THE ACIDURIC GROUP OF BACILLI

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A generally accepted characteristic of the bacilli grouped together as aciduric or acidophilic, such as the bacillus bulgaricus, the bacillus acidophilus, the Boas-Oppler bacillus and others, has been the absence of gas production when grown in sugar media. Recently one[1] of us noted, in the course of the routine isolation of aciduric bacilli from stools, that a very few produced gas. We further found that when human feces are planted into N/20 acetic acid glucose broth, in some instances there may be gas production without the presence of yeasts. Further investigation revealed the fact that the gas was formed by a bacillus of the same morphology, staining, and general biologic properties as the bacillus acidophilus. Since a careful search of the literature has not disclosed a description of a bacillus of this type, we have considered it a new species and have named it Bacillus acidophil-aerogenes.

To obtain some idea of the frequency with which this bacillus occurs in human feces, 38 specimens from 37 individuals were examined, of which the majority were from very young infants. The feces of 9 individuals yielded this bacillus, of which 7 were adults and 2 infants, one of the latter being 8 days old and the other 6 months. Of 24 very young infants, ranging in age up to 19 days, in only one instance was the bacillus acidophilus found in the stool. On the other hand, a systematic examination of a few adult stools revealed its presence with only one exception. It seems probable that it occurs in the intestinal tract of most individuals and usually in much smaller numbers than does the non-gas-producing bacillus acidophilus of Moro.

This same aciduric gas-producing bacillus was also isolated readily from the feces of the sheep and the hen, but not from the few fecal specimens examined from dog, monkey, rabbit, guinea-pig, white rat, white mouse, goose, and pigeon. It is our belief, however, that the bacillus is widely distributed in nature and that an intensive search would reveal it in the intestinal tracts of a wide variety of animals.

In the isolation of this bacillus from the feces, a small amount of the material is seeded into +5.0 acetic acid glucose broth in fermenta-

1. Rahe: Jour. Infect. Dis., 1914, 15, p. 141.

tion tubes and incubated from 2 to 3 days. The tubes containing this gas producing aciduric bacillus are then readily recognized. If the gas is due to yeasts, the fact will be indicated by the characteristic pellicle formation. This preliminary culture is then streaked on glucose oleate agar and several colonies are seeded again into the acetic acid broth, incubated for 2 days, and transferred to glucose broth tubes in which the natural acidity conferred by the meat has not been modified.

Either type of colony described by Mereschowsky for the bacillus acidophilus group may be produced by the bacilli of this type. That is to say, certain strains of this bacillus, when plated out on glucose oleate agar, formed small, round, white, opaque colonies about 1.2 mm. in diameter (Type 1), while other strains formed colonies that are gray, semi-transparent, smaller than a pinhead, with short, tooth-shaped projections (Type 2). After a few generations on oleate agar, this bacillus was induced to grow on glycerin agar of about +1.0 reaction and also on sugar-free agar of the same degree of acidity. The growth on these media was discrete and generally invisible before the second day. The colonies resembled those of streptococcus.

Altho, as a rule, as seen in fecal smears and on first isolation, the bacillus acidophilus is a slenderer rod than the bacillus bulgaricus, it exhibits a marked tendency to vary in morphology, and, after a few generations on artificial media, certain strains may assume a size and morphology which are identical with those of the bacillus bulgaricus. A typical strain of this gas-producing variety, grown 24 hours on unneutralized glucose broth, developed strongly gram-positive bacilli, in length from 1.50 to 11.50 microns and in width about 0.80 microns. Altho the greater number of the bacilli averaged about 5 microns in length, strings up to 40 microns were present in almost every field. With Loeffler's alkaline methylene blue, the bacilli stained evenly. On glycerin agar, the bacilli were thicker and longer than those grown in sugar broths or on oleate agar. Occasional Y-forms were to be seen, and the strings were frequent, long and curved.

These gas-producing aciduric bacilli grow vigorously in sugar broths of proper acidity and very characteristically. The type of the bacillus which does not produce gas tended to cloud the medium evenly and rather lightly, whereas the gas-producing type formed a growth showing a marked tendency to adhere to the bottom and the side of the tube; but, if the latter culture was shaken, the medium became as

heavily clouded as in the case of the bacillus bulgaricus. Like the bacillus of Moro, this gas-producing type was not motile. As regards the rate of growth in fluid media, it was found that this gas-producing type multiplied much more rapidly than did the bacillus bulgaricus or the bacillus acidophilus (Moro). In unneutralized glucose broth, B. acidophil-aerogenes gave rise, 7 hours after seeding, to a distinct cloud of growth, whereas the control cultures of the bacillus bulgaricus and the bacillus acidophilus (Moro) showed no evidence of growth.

This bacillus was found to ferment actively, at 37 C., the following carbohydrates: maltose, saccharose, lactose, raffinose, and dextrose. For these tests, unneutralized, sugar-free broth was used as a base. No growth occurred in mannite or dextrin broths and none in any medium at room temperature. B. acidophil-aerogenes forms acid more actively on the average in sugar media than does the bacillus bulgaricus or the bacillus acidophilus (Moro). With 16 cultures of B. acidophil-aerogenes, grown for 3 days in unneutralized dextrose broth, the average percentage of normal acid was 10.0; whereas with 12 strains of the bacillus bulgaricus,[1] incubated for 5 days, it amounted to 5.61, for 21 strains of the bacillus acidophilus Group 2 (Rahe), to 5.66, and for 20 strains of the bacillus acidophilus, Group 3 (Rahe), to 5.09. The acid production of B. acidophil-aerogenes in terms of lactic acid is detailed in the following table.

TABLE 1

Acid Produced in 2% Glucose Broth in Terms of Lactic Acid (Incubation 72 Hours at 37 C.)

Jam.	0.945	Baby-3	1.125	C-5	0.720
B-2	1.220	Baby-1	1.080	H	0.765
Ca-1	0.810	Buck	0.855	C-3	0.810
Be-9	0.855	Ca-3	0.855	Ca-4	0.720
Case-3	0.720				
C-2	0.810				
I	1.170				
C-6	0.765				

The essential characteristic distinguishing this bacillus from the other hitherto described aciduric bacilli is the production of gas. This was produced, almost without exception, in broth containing any one of the five sugars mentioned. The amount of gas produced varied greatly for the several strains tested and even from time to time for a single strain. No increase in the amount of gas produced followed any increase in the amount of carbohydrate above 1%. Frequently an almost complete resorption of the gas occurred. After 72 hours' incubation, the maximal amount formed with any strain was 60% and the minimal 2%. For 16 cultures, the average amount formed in dextrose was 23%. The greater part of this gas was always hydrogen, the formula varying from 4H : 1 CO_2 to 6H : 1 CO_2.

Curiously enough, altho these gas-producing aciduric bacilli split lactose actively with a large production of acid, they grow poorly or not at all in milk. Only 1 strain clotted milk as early as 72 hours, while with 13 strains even partial clotting did not occur for from 15 to 20 days. Six cultures had no apparent effect upon the milk medium. The clot, when formed, was soft, with only a slight separation of whey. There were no gas streaks, even on continued incubation. In an effort to adapt these bacilli to a milk medium, several strains were replanted in this medium at 48-hour intervals for 5 weeks, but with no shortening in the time required to clot.

As is well known, the members of the aciduric group soon die out if kept at incubator temperature, on account of the increasing concentration of acid. In a comparative test, it was determined that a strain of the bacillus bulgaricus survived 6 days at 37 C. when growing in the glucose broth medium, while the bacillus acidophilus (Moro) lived for 15 days and B. acidophil-aerogenes for 8 days. At room temperature, however, B. acidophil-aerogenes survived much longer than did the other two types. At the end of 37 days, B. acidophil-aerogenes was still alive, whereas the bacillus acidophilus (Moro) had died out after 15 days and the bacillus bulgaricus after 6 days. At ice-box temperature, all the cultures were alive at the end of 50 days, altho the viable bacilli had decreased greatly. In the last two tests, the cultures were grown for 48 hours at 37 C., and then placed in the dark at the respective temperatures.

We have found that an agglutinating serum for the bacillus acidophilus may be produced without difficulty. Rabbits were inoculated intravenously with the growth from glycerin agar cultures. After 3 or 4 injections a titer as high as 1:800 was obtained. Of 14 strains of B. acidophil-aerogenes tested against a serum immune to one member of this gas-producing group, 10 gave positive agglutinations. On the other hand, none of several strains of the bacillus acidophilus of Moro reacted to this serum. Contrariwise, a serum immune to a strain of the bacillus acidophilus of Moro did not clump any of a number of strains of the gas-producing type. Further experiments of this character seemed to indicate that the gas-producing group of aciduric bacilli exhibited a higher degree of homogeneity than did the several strains conforming to the type of the bacillus acidophilus of Moro.

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A STUDY OF THE SO-CALLED IMPLANTATION OF THE BACILLUS BULGARICUS

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After Metchnikoff's announcement that certain lactic acid-forming bacilli found in the fermented milk Yahourth were able to check the growth of putrefactive bacteria in the intestines, in all the articles published in attempted confirmation of his claims, the methods used to identify the bacillus are inadequately described. This work was undertaken with the purpose of determining, in the light of a better understanding of the cultural peculiarities of the bacillus bulgaricus, whether or not a true implantation took place.

In the human intestine there occur normally bacilli that culturally and morphologically resemble this bacillus very closely. The group of organisms, which includes the bacillus bulgaricus, is distinguished from other groups of bacteria by the ability of its members to grow in media containing considerable amounts of acid; because of this characteristic, these bacilli are called aciduric or acid-tolerant organisms. In a previous paper[1] the writer was able to show that, contrary to the usual statement, these bacteria grow luxuriantly in an ordinary laboratory medium, viz., *unneutralized* meat-peptone broth containing glucose or other suitable carbohydrate, and that agar prepared from this broth, with or without the addition of 0.2 percent of sodium oleate, is an excellent solid medium. It was also shown that while the bacillus bulgaricus, from its cultural and perhaps biochemical properties, also belongs to this group of sugar fermenting, acid-tolerating organisms, it differs from the bacteria of the type of the bacillus acidophilus in failing to ferment maltose. Some strains do not attack saccharose.

REVIEW OF THE LITERATURE

Bertrand and Duchacek[2] mention the failure of this organism to ferment maltose, but do not give the nature of the medium used. On the other hand

1. Jour. Infect. Dis., 1914, 15, p. 141.
2. Ann. de l'Inst. Pasteur, 1909, 23, p. 402.
3. Rev. méd. de la Suisse romande, 1905, 25, p. 714.
4. Compt. rend Soc. de Biol., 1906, 60, p. 558.

Grigoroff and Cohendy,[4] equally indefinite as to medium, claim an opposite result. This contradiction in evidence leads to the suspicion that faulty identification may have led, in at least some instances, to erroneous conclusions as to the implantation and survival of this bacillus in the intestine. Cohendy claims to have demonstrated that the organism becomes established in the intestine in eight days and survives for twelve days or less after feeding has been stopped. According to Belanowsky[5] the bacillus becomes adapted to the human

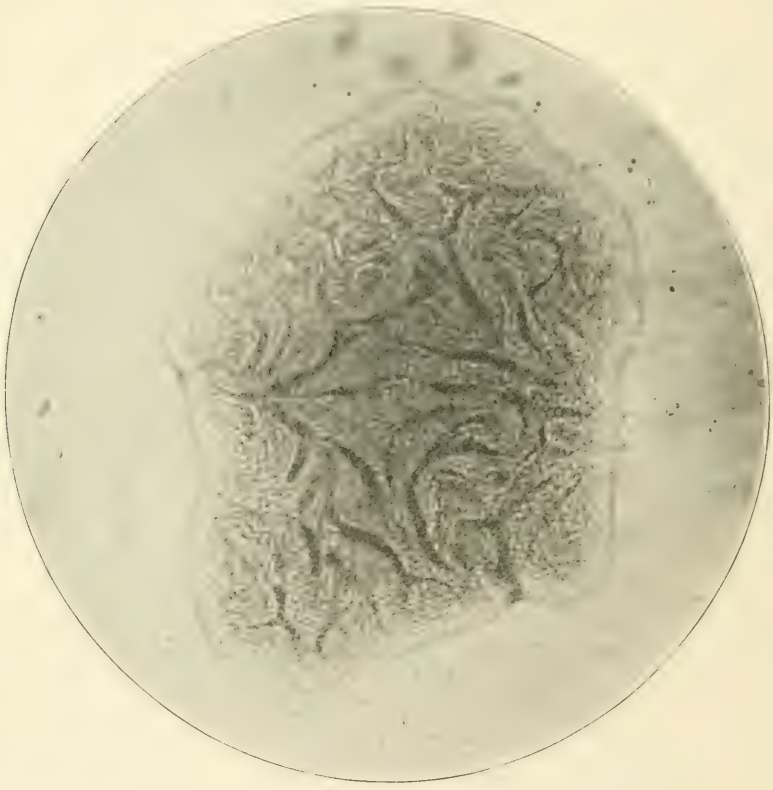

Fig. 1.—An atypical colony of the bacillus bulgaricus. No projections. × 60.

intestine and survives for varying periods. Distaso and Schiller[6] fed the organism to rats and concluded that it is impossible to force the growth of a foreign organism in the intestine. Herter and Kendall[7] fed to monkeys Bacillac, a commercial fermented milk in which the bacillus did not occur in pure culture. The organism was recovered from the stools on the fifth day after feeding commenced. After fourteen days of exclusive feeding with this milk the organism was found in almost pure culture in the duodenum and jejunum, in

5. Ann. de l'Inst. Pasteur, 1907, 21, p. 991.
6. Compt. rend. Soc. de Biol., 1914, 66, p. 243.
7. Jour. Biol. Chem., 1908, 5, p. 293.

less numbers in the cecum, and hardly at all in the colon and rectum. In a personal communication to the writer, Dr. Kendall has described the method of isolation used in his investigation. A small amount of feces was seeded into milk and pure cultures obtained by repeated transfer in this medium. The organism was recognized by its complete parasitization to milk.

It seems certain that Herter and Kendall succeeded in isolating the organism ingested, but in the case of other workers the lack of

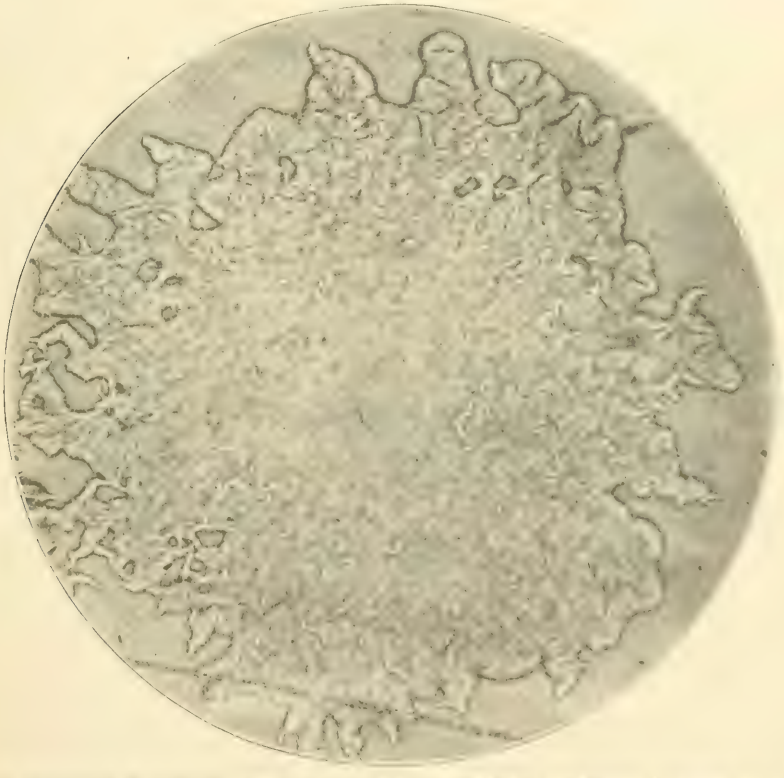

Fig. 2.—Colony of intestinal isolans bacteria simulating that of the bacillus [illegible]

cultural detail leaves one in doubt as to the correctness of their findings.

For the identification of the bacillus bulgaricus colony formation, morphology and quantitative estimation of acid production are insufficient. The colonies are usually described as resembling those of the bacillus anthracis in their loose texture, irregular outline, and forma-

tion of projection. While this so-called characteristic form is frequent, a more regular form, one that resembles the typical form only in its fissured surface, is also common (Fig. 1). During the course of this work there often occurred on the plates, made from the enrichment tubes which had been planted with feces, colonies of aciduric bacteria normal to the intestine that very closely resembled those of the bacillus bulgaricus (Figs. 2 and 3). During the period of ingestion

Fig. 3.—Colonies of the bacillus acidophilus with projections. About × 6.

mixed colonies, composed of the bacillus bulgaricus and intestinal acid-tolerant bacilli, were common. In staining and shape the bacillus bulgaricus is identical with the bacillus acidophilus.

While some strains of the Bulgarian bacillus are capable of a high acid production, others do not produce so great an acidity as that formed by some strains of the bacillus acidophilus. The inability of the bacillus bulgaricus to utilize maltose constitutes its essential difference from the intestinal aciduric bacteria that coagulate milk.

In this investigation pure forty-eight-hour cultures of the bacillus bulgaricus were ingested in the quantities indicated on the charts. Stools were collected at regular daily intervals and treated as follows: Within a few minutes after the passage of the movement a representative sample of 0.5 gm. was accurately weighed and emulsified in 50 c.c. of normal salt solution. This suspension was shaken for three minutes and the grosser particles allowed to settle. A series of dilutions was prepared and 0.5 c.c. of each dilution was seeded into tubes containing 10 c.c. of milk having an acidity of plus 2.5 percent normal lactic acid. Milk, acidified or not, has been used as an enrichment medium by practically all of the workers in this field. After six days' incubation at 37 C. a loopful from each milk tube was streaked on the surface of hardened and dried meat-peptone-oleate-glucose agar. After forty-eight hours' incubation likely colonies were fished and maintained in the unneutralized glucose broth.

On the plates from the enrichment tubes there were, as might be expected, mixed colonies composed of intestinal aciduric organisms and the bacillus bulgaricus, but the latter could not be distinguished as such under the microscope. In most cases cultures from such mixed colonies gave an acidity in milk nearly as great as that of the pure cultures of the organism ingested. Growth in maltose broth showed the presence of intestinal organisms and plates made from the primary cultures in glucose broth invariably showed colonies of both organisms, while plates from the maltose broth showed colonies of bacilli only of the acidophilic type. Occasionally in the mixed cultures in milk the intestinal organism predominated and the acidity did not rise above plus 17.0 percent normal in six days.

An attempt was made not only to determine whether the organism ingested could survive in the intestine but also to determine to what extent it was present. To that end, in addition to the monkeys, human subjects presenting varying intestinal conditions were chosen. In all of the experiments that follow the culture was taken just before meals.

Subject A, female, 32 years old, in the third or fourth month of pregnancy. The stools were typically putrefactive. There was only a slight constipation. The average daily diet of Subjects A and C was as follows: fruit 407 gm., bread 147 gm., cereal 158 gm., meat 230 gm., vegetable 280 gm., cane sugar 27 gm., milk 100 c.c., dessert (bread pudding, tapioca, blanc-mange) 142 gm.

Chart 1 shows that the organism appeared in the stools of Subject A for the first time on the eighth day. For the period during which broth culture was fed the curve reaches its greatest height on the tenth day and this point is not passed during the increased ingestion in the incompletely recorded period of broth culture feeding that follows. With the milk culture the excretion reaches its greatest height, but

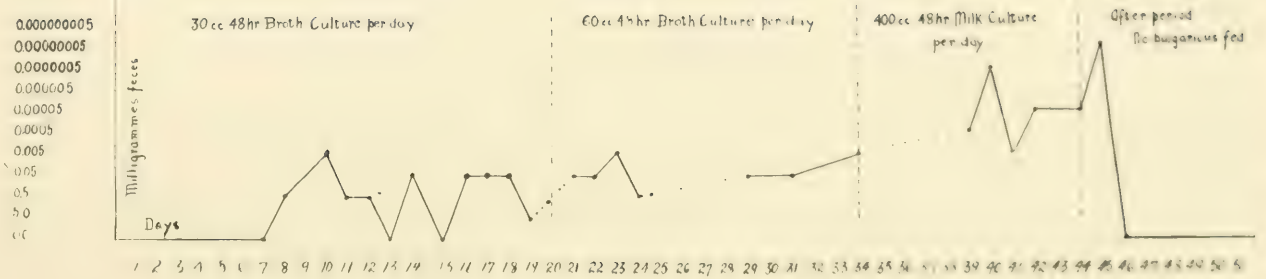

Chart 1 (Subject A). Greatest excretion of the bacillus bulgaricus reached on day after feeding stopped

the greatest elimination occurs on the day after feeding was stopped. The bacillus disappeared from the stools on the following day.

Subject B, male, aged 53. He subsisted both before and during the experiment entirely upon vegetable food. His stools were dry, almost odorless, and characterized by their high content of aciduric bacteria. One liter of a forty-eight-hour milk culture was taken per day. The bacillus bulgaricus appeared in the feces for the first time on the fifth day and the greatest excretion was reached on the sixth day. After its first appearance the organism was absent from two consecutive stools on two occasions. It maintained its level for one day after the feeding stopped and disappeared on the third day after the last ingestion.

The organism was fed to Subject B in enormous numbers. Altho diet and intestinal conditions were such as would be expected to favor its development, and the bacillus appeared in the stools three days

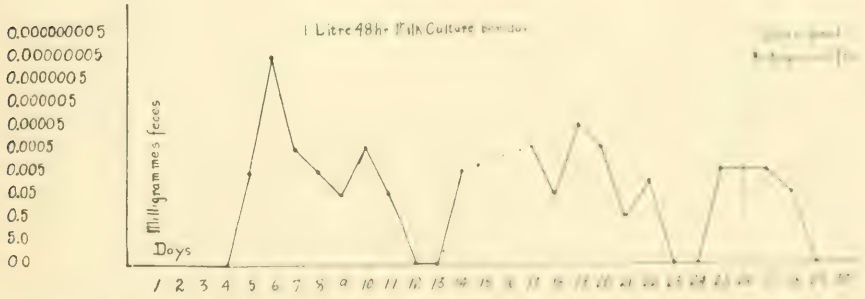

Chart 2 (Subject B).—Ingested organism absent on third day after feeding stopped.

earlier than in the preceding experiment, it survived but one day longer.

Subject C, male, 32 years old. The diet was of the same quantity and composition as that of Subject A. The feces were moderately putrefactive. The organism appeared on the second day following the first ingestion and survived for the same length of time as in the case of Subject B. As in the other two subjects, this bacillus disappeared completely from the stools within a few days after the ingestion of the culture was stopped and was not recovered again, altho the examinations were continued daily for a week. In one instance during the feeding of the culture the bacillus was absent from three consecutive stools.

Neither in this nor in the preceding experiments did the disappearance of the organism occur at times when several stools were passed during two days. Their absence must have been due to some other cause than a sudden elimination. Either the bacillus died off rapidly

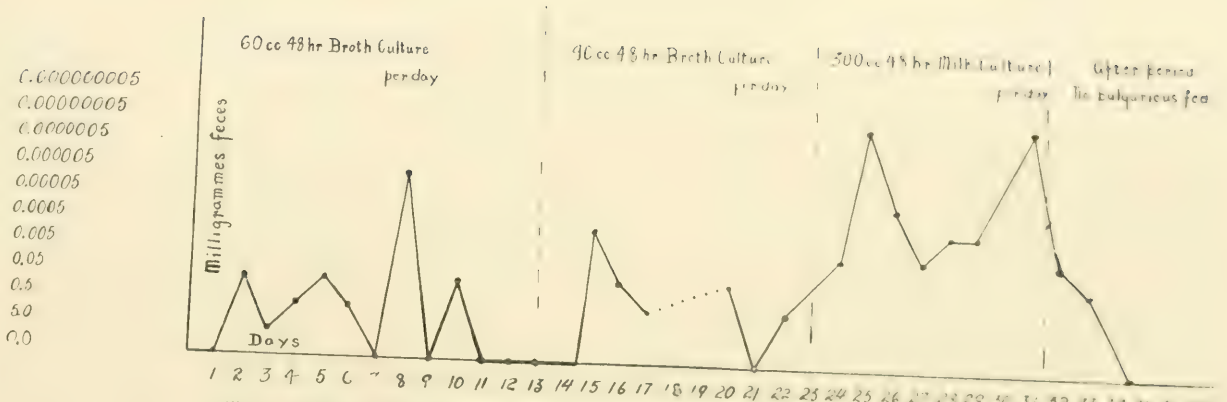

Chart 3 (Subject C).—Ingested organism absent on third day after feeding stopped.

or it was retained in the upper levels of the digestive tube and passed into the feces in numbers too small to be detected.

Subject W., female, 25 years old, had been taking the bacillus bulgaricus in tablets for several months. For eight weeks during which this subject received 60 c.c. of broth culture per day the stools were examined at irregular intervals. The organism was found present in nearly all of the stools tested and survived eight days after feeding stopped.

Altho in the above experiments the bacillus bulgaricus disappeared from the feces soon after ingestion stopped, it has not been proved that the organism does not survive for a longer period in the small intestine. It is possible that an organism might be able to adapt itself to the conditions prevailing in the duodenum and jejunum, but be unable to survive in the lower intestine, and so be absent from the stools.

In order to determine if such survival occurred, small Rhesus monkeys that had been fed the bacillus bulgaricus were killed at different intervals after the feeding was discontinued. Five-tenths of a gram of the contents of the different regions of the intestine were treated in the same way as the feces of the human subjects. The monkeys were fed twice a day and received the following average diet: one banana, 99 gm. boiled rice, one apple, 58 gm. bread, 62 gm. cabbage.

Monkey 1 was given fifty lactose tablets per day, each of which contained approximately 2,000 bacilli. The tablets were prepared as follows: after a heavy broth culture was centrifugalized, the deposited organisms were suspended in a very small amount of salt solution, and with lactose were formed into tablets. Fresh forty-eight-hour cultures were used and the tablets fed just before meals. After the thirteenth day the feeding was stopped and the animal killed on the day following. The bacillus bulgaricus was recovered in fairly large numbers from the jejunum, but not from the other parts of the digestive tube and feces.

Monkey 2 was fed 300 c.c. of forty-eight-hour milk culture per day for six days and cultured seven hours after the last feeding. The organism was found in large numbers in the duodenum, jejunum, ileum, and feces, and very scantily in the colon.

Monkey 3 received the same amount of culture for the same length of time as Monkey 2, but the feeding was stopped seven days before death. The bacillus was recovered in small numbers and only from the duodenum.

This series of experiments, while not complete, serves to show that the organism may survive in the small intestine after it has disappeared from the lower digestive tract and feces, tho it is probable that this survival is not permanent. Table 1 gives the results of these experiments.

Altho it is probable that the bacillus bulgaricus once flourished in the intestine of a warm blooded animal, as its preference for body temperature shows, the facts brought out in this investigation indicate that it is no longer capable of developing in the lower part of the digestive tube. Its absence from the feces, unless previously ingested in great numbers, is especially interesting in view of the fact that Hastings and Hammer[8] have discovered an organism resembling the Bulgarian bacillus in milk, butter, and cheese. It is probable that their bacillus was an intestinal organism, but, since these authors do not state whether it attacks maltose, it is probable that they were dealing with an exceptionally active strain of the bacillus acidophilus.

TABLE 1

THE SURVIVAL OF THE BACILLUS BULGARICUS IN THE INTESTINES OF MONKEYS

Monkey	Fed	Period of Ingestion	Time After Last Ingestion	Duodenum	Jejunum	Ileum	Colon	Feces
1.....	Fifty tablets per day.....	13 days	24 hrs.	—	+	—	—	—
2.....	300 c.c. 48-hr. milk culture..	6 days	7 hrs.	+	+	+	+	+
3.....	300 c.c. 48-hr. milk culture..	6 days	7 days	+	—	—	—	—
Control	Not fed.....	.....	.....	—	—	—	—	—

The sign + denotes the presence of the bacillus bulgaricus; the sign — denotes absence.

The mere recovery of an organism from the feces several days after the feeding has stopped does not prove that it has become adapted to the intestine, and, while the evidence developed in this investigation does not exclude the possibility of a slight multiplication of the bacillus bulgaricus in the lower part of the human digestive tract, it is obvious that nothing resembling a true implantation took place.

The charts show that the point of greatest excretion of the ingested organism is the same for all three subjects and represents an elimination of at least twenty million organisms per milligram feces. The average daily excretion for Subjects A, B, and C was about one million organisms per milligram.

Torrey,[9] in an investigation of the fecal flora of typhoid patients on a high calory diet, found that the excretion of bacteria of the type of the bacillus acidophilus frequently reached one million organisms per milligram feces and in one instance was six times that amount.

8. Univ. of Wis. Agric. Exper. Sta. Research Bull. No. 6, 1909, p. 195.
9. Jour. Infect. Dis., 1915, 16, p. 72.

In the present investigation intestinal aciduric organisms were frequently encountered at very high dilutions of the feces and it is probable that their excretion paralleled if it did not exceed that of the bacillus bulgaricus, notwithstanding the enormous ingestion of the latter.

CONCLUSIONS

The bacillus bulgaricus is an organism readily distinguished from the intestinal aciduric bacteria.

The evidence indicates that this bacillus cannot become adapted to the human lower intestine.

The experiments with monkeys show that the bacillus bulgaricus is capable of an apparently limited survival in the upper intestine of these animals.

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A METHOD OF STUDYING THE EFFECT OF SERUM UPON TISSUES.

BY S. FELDSTEIN, M.D.

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In the investigation of the action of serum ferments on animal tissues chemical methods have been almost exclusively used. Within the last few years the dialysis method developed by Abderhalden has been extensively employed in experimental as well as clinical investigations. While the technique of this method is not as difficult as it is usually assumed to be, at every step of the procedure a not inconsiderable number of errors are likely to creep in. It requires very careful preparation of the substrate, absolutely uniform dialyzing tubes and great care in boiling the dialysate.

The most weighty objection to the dialysis method, however, lies in the fact that it gives only indirect evidence of the enzymatic action of the serum on the tissue. It is due to this fact that recently Bronfenbrenner and others have claimed that a positive Abderhalden test does not show the proteolytic action of the serum on the tissue substrate at all, but that during the incubation of the serum with tissue, the antiferment of the serum becomes absorbed. The removal of the antiferment exposes the serum proteins to the action of the ferments in the serum and it is their cleavage products which pass into the dialysate and give the positive reaction.

Considerations of this nature led the author more than a year ago to seek for a method of demonstrating the ferment action of serum in the histologic examination of tissues. A priori it was to be expected that early evidence of the chemical changes resulting from ferment action might be shown by alteration in the microscopic appearance and staining reaction of the tissue.

Almost the first specimen examined, that of a boiled guinea-pig placenta exposed to the action of normal and pregnant guinea-pig serum, having fortunately shown a most remarkable difference,

the author was encouraged to continue the investigation. Soon it became evident that the histologic method was so delicate an indicator that unmistakable evidence of the action of normal serum on animal tissue was readily demonstrable. The activity of the serum varies greatly with the species of animal from which the serum is derived. Of those investigated the serum of the cat is apparently one of the most powerful and it is with the normal serum of this animal that the present investigation was chiefly concerned.

The serum was obtained from the jugular vein and immediately centrifuged; in this manner best avoiding the mechanical hemolysis which is likely to occur. At first, in addition to the boiled tissue, we attempted to use formalin and acetone fixed sections but found that these fixatives almost entirely inhibited the action of the serum.

In our more recent work we have used fresh and boiled tissue almost exclusively. The use of fresh tissue theoretically did not promise much, as, on the authority of Abderhalden, it has long been assured that only in the boiled state do the tissue proteins undergo digestion. Moreover, the work of Longcope and others has shown that serum has a preservative action on tissue. This author, however, calls attention to the fact that in the most superficial layers of the block of tissue immersed in serum, the nuclei do not stain, and it is apparently these changes that we are concerned with in our present investigation. In the use of fresh tissue, the factor of autolysis is involved. But as Launoy has shown with the guinea-pig liver, and as we have been able to confirm, marked histologic evidence of autolysis does not appear within the first 24 hours, if the temperature is kept below 37.5 degrees C. Our sections were incubated from 18-21 hours, and in only a few instances did our controls show marked autolytic changes, when needless to say that particular experiment was rejected.

When boiled tissue was used as substrate, small blocks about 2 cm. in thickness were cut, washed in water, then thrown in physiological salt solution and boiled for fifteen minutes. Frozen sections, 10 microms in thickness, were made of the fresh and boiled organs.

The activity of the serum was determined by a series of dilutions, 1, 1/10, 1/20, etc., in physiological salt solution.

The sections (all of about the same size and thickness) were placed in small test tubes, each containing 1 c.c. of the serum dilution. 1 c.c. of physiological salt solution was the constant control. To each tube was added 1 cc. of toluol and the whole series incubated at 37.5° C. for 18-21 hours.

The staining was done by means of Delafield's hematoxylen and eosin. All sections of a series were stained in an identical

Delafield's hematoxylen	1 min.
½ per cent. alcohol eosin	10 sec.
85 per cent, alcohol	30 sec.
Oil of origanum	Until clear.
Canada balsam mounting.	

manner. Fresh sections were treated by floating on a slide, drying on blotting paper over the paraffin stove, then staining. Boiled sections were transferred in the moist state to the various solutions, as otherwise they are likely to float off the slide. We have also employed the various fat stains and the Altmann granule stain but have found the simple hematoxylen-eosin adequate.

The most striking changes are found in the nuclei. When exposed to the action of cat serum up to a dilution of 1 : 30, no nuclei are to be seen in the boiled sections. On fresh tissue, contrary to what might have been expected, this action was even more marked, as no nuclei were visable at a dilution of 1 : 120 of the serum. The nuclei either had disappeared entirely or had failed to take the stain. In addition the tissue appeared cloudy, parboiled, and not infrequently cleavage lines ran through the whole specimen. At times, as in the guinea-pig placenta to be shown on the screen, the organ assumed a reticular structure, as inspecimens treated by the Spalteholz or Mall's digestion method. At other times, the whole specimen appeared homogeneous, the tissue becoming unrecognizable. Fibrous tissue as the trabeculæ of the spleen and adventitia of vessels stand out distinctly, but the nuclei seem to have disappeared. The appearances were very similar to those observed in autolysis of organs.

When we had established that the titre of activity of cat serum remains constant, *i. e.*, no nuclei in the boiled sections on exposure to a dilution of 1 : 30 or 1 : 40 we injected a cat whose serum was previously tested, with 1 gm. of fresh cat liver freed from blood, intraperitoneally three times, and tested its serum against cat

liver. The action of the serum after the series of injections had increased so that the specimens exposed to a dilution of 1 : 60 resembled histologically the ones previously exposed to 1 : 30, etc. The action on the spleen was not nearly as marked.

The serum of a rabbit, injected with Beebe's nucleoproteids derived from dog thyroid, at a dilution of 1 : 5 had a much more striking action on the fresh dog thyroid than on the dog spleen.

As to the exact interpretation of these changes: They cannot be attributed to bacterial action as toluol was always added in abundant amounts and we have found that not infrequently bacteria failed to grow on culture media when they were kept in the same incubator owing to the evaporation of the toluol. Moreover, in a series examined at intervals of ten minutes, we have found that at the end of an hour, the changes in the histologic appearance of the sections were quite marked; a period of time too short for bacterial action to have taken place. We have also been able to show that while inactivation at 55° C. for one hour failed to destroy this activity of the serum, exposure to 65° C. for one half hour led to complete loss of activity.

From the appearance of the specimens, it is my belief that a number of ferments might be concerned in the production of these striking changes, not only proteases but also peptases and perhaps esterases and nucleases. Sodium fluoride in 0.3 per cent. solution, which is a specific inhibitor of esterase action seemed to weaken the activity of the serum. With normal serum extracted by chloroform according to the method advised recently by Jobling, we have not obtained uniform results. When the serum after filtration remained turbid, as was often the case, no action on the tissue was demonstrable. This activity of the serum is apparently not identical with that of trypsin. With the latter, there is marked fragmentation of the sections at a time when nuclei are still visible. While the present investigation was in progress, there appeared a brief preliminary communication in The *Muenchener medicinische Wochenschrift*, No. 37, 1914, by H. Rollett describing a method by which bits of boiled placenta were exposed to serum, then examined histologically. Very meager details are given.

Dr. W. L. Rost has assisted me in most of the experiments of the present investigation.

THE INCISION OF TUMORS FOR DIAGNOSIS.

By James Ewing, M.D.,
New York.

The essential basis of the competent treatment of malignant tumors is a full knowledge of their point of origin, their pathological nature, and their natural history. As this knowledge becomes more and more comprehensive, each malignant tumor asumes the character of a specific disease. The modern conception of tumors requires one to distinguish between rodent ulcer of the skin and epidermoid carcinoma of the tongue just as sharply as between eczema and psoriasis. Both these tumors are carcinomas of the surface epithelium, but they arise under wholly different conditions, pursue divergent courses, and present quite different therapeutic problems. They are specific clinical and pathological entities, quite as much as are eczema and psoriasis. It can no longer be regarded as a sufficient diagnosis to pronounce a tumor to be simply carcinoma or sarcoma. In fact, such a partial diagnosis may be without any practical meaning whatever, since some processes to which these terms are attached are benign and self limiting, while others are malignant and invariably fatal.

When the tissue of origin is added to the general morphological diagnosis of a tumor, the information conveyed is vastly increased. A carcinoma of the tongue at once suggests a very different problem from that of carcinoma of the skin, and in the minds of surgeons and pathologists the category of periosteal sarcoma is far more formidable than that of benign epulis.

Yet the determination of the exact tissue of origin is quite incompetent to reveal the practical significance of the tumor process. In each organ there are many variations in the natural history of carcinomas and sarcomas which the surgeon must recognize before he can grasp the significance of the condition and design appropriate treatment.

Thus the giant cell sarcoma of the tendon sheaths is a benign process, whereas giant cell periosteal sarcoma arising in the same region is a highly malignant and dangerouus disease. A papilloma or papillary carcinoma of the tongue pursues a very different course from that of infiltrating carcinoma of this organ. It is quite possible that a very experienced clinician may accurately estimate the nature and origin of a tumor and its natural history in an ordinary clinical examination, and it is highly desirable that this type of clinical judgment should be developed to the highest degree, but at present it is a matter of universal experience that clinical diagnosis receives invaluable aid by supporting its conclusions with the microscopical study of tumor tissue. Indeed so definite and decisive is the evidence furnished in many instances by microscopical study that the practice of resting the diagnosis

Hodgkin's disease may be made from a tumor removed from the spinal canal, and correct diagnosis of gastric adenoma has been based on an intrascapular metastasis, but in most cases accurate clinical data are necessary and often essential for any significant interpretation of microscopical findings.

4. The prognosis of a tumor may to a considerable extent be based on the microscopical structure. This assertion may be successfully maintained just to the extent that the pathologist is able to interpret the clinical diagnosis from the microscopic section. There are numerous instances in which such a diagnosis is possible and among them may be mentioned Hodgkin's granuloma, malignant lymphosarcoma, chorio-carcinoma, embryonal carcinoma of the testis, carcinoma of thyroid, osteogenic sarcoma of humerus, femur, or trunk, and many other tumors of which the prognosis is practically fatal.

In another group of cases the potential malignancy of the tumor may be judged with much accuracy from the histological structure, but its actual clinical course may be subject to wide variations, depending chiefly on the age of the patient, the location of the tumor, and its size and duration. The accuracy of this type of diagnosis will depend chiefly on the experience of the pathologist and the clinical data at disposal. While errors in judgment resulting from experience must doubtless occur in this field, I am heartily in favor of attempting this type of diagnosis wherever possible. In mammary cancer, for example, it is highly important to distinguish between comparatively benign papillary adeno-carcinoma, which carries a good prognosis, and true alveolar carcinoma, which in young subjects is nearly always fatal. Each organ presents its own scale of potential malignancy for the various morphological types of carcinoma. In my own experience I have to acknowledge errors in judging the potential malignancy of some giant cell sarcomas of bone, but these errors were due to inexperience, and not, as has been somewhat widely proclaimed, to lack of relation between the histological signs of malignancy and the clinical course of the tumor. In rare instances highly atypical tumors with pronounced histological signs of malignancy fail to display malignant clinical features, but these cases are rare and I see no reason why they should not be catalogued and called to the attention of microscopists and surgeons.

5. The use of frozen sections, while occasionally of decisive value, encourages hasty conclusions and readily leads to error. It is probably most often employed in operations on the breast where it is very prone to mislead. It should be replaced as far as possible by the gross examination of the whole tumor, which in the great majority of cases yields signs of malignancy or of benign qualities which are quite as conclusive as are microscopical pictures. The knowledge of the gross diagnosis of tumors is a much neglected field the development of which is retarded by reliance on frozen sections. Most pathologists who are frequently called upon for frozen sections, make their diagnosis on the gross appearance of the tissue and use the frozen section for confirmation. Where the gross appearance leaves doubt, the frozen section usually strengthens the doubt and I have known it distinctly to mislead when suggesting a con-

clusion contrary to the gross diagnosis. The gross examination proceeds under the great advantage of including the whole mass of tissue submitted, whereas the frozen section can apply only to one portion or successive portions, the choice of which depends wholly on the examiner's capacity to recognize carcinoma in the gross. In the absence of wholly characteristic clinical and gross anatomical signs of malignancy it is unwise to risk the sacrifice of important structures, such as half of the tongue or a limb, without resorting to microscopic section. With the modern improvements in technic the frozen section often furnishes a prompt and trustworthy decision, but when the structure of the tumor is atypical, more time should be given for a deliberate study.

6. No rigid rules can be safely followed in deciding when to remove a portion of a tumor for diagnosis. The conditions surrounding the growth of tumors are so variable that each tissue and organ must be considered by itself. It may therefore be profitable to consider in detail some of the more common situations where the question of microscopic diagnosis arises.

Skin. Superficial elevated, warty, or ulcerating tumors of the skin, or inflammatory processes suggesting tumors, may I believe be safely subjected to the trauma of incision. In most cases the establishment of the exact nature of the process is far more important to the patient than the inconvenience of a slight operation. The tissue should include the edge of the lesion, with a border of normal skin, and the derma. Pigmented moles and all plain or suspected cases of melanoma form a peremptory exception to this rule, and should not be touched except by liberal excision. Small rodent ulcers are also extremely dangerous when narrowly excised.

Lip. There appears to be little excuse for cutting into small early epidermoid cancers of the lip, which should be recognized in the gross and completely excised or otherwise treated. When the lesion is larger and ulcerating, a minute portion of tissue may safely be sacrificed for diagnosis.

Buccal and lingual mucosa. The necessity for microscopical diagnosis of ulcerating lesions of the mouth, pharynx, and tongue often arises, and the careful removal, without crushing, of a small portion of the edge of the ulcer seems permissible. In deference to contrary opinions extreme care in the operation must be urged.

Esophagus. Janeway has devised an instrument by which a small fragment of tumor may be removed through the esophagoscope, and in a series of cases he has been able to establish the diagnosis of some very early as well as of advanced lesions.

Larynx. The early diagnosis of carcinoma and other lesions of the larynx is often accomplished by examination of a small fragment snipped by a suitable cutting forceps. This procedure does not appear to aggravate the disease, but several sections are sometimes required to locate the tumor.

Stomach. By gastroscopic examination, carcinoma or ulcer of the stomach may be located and a small portion of the affected tissue may be safely removed by an instrument devised for this purpose. Although the procedure is not without hazard, the injury to the stomach by the removal of tissue does not appear to be any contraindication.

Rectum. The character of polypoid or ulcerating tumors of the rectum may be safely determined from portions of tissue removed through a speculum, but incisions into hard cancerous strictures should be avoided.

Bladder. Villous papilloma of the bladder is often identified from fragments of the tumor found in the urine or in washings, or removed with the aid of the cystoscope. Carcinomatous fragments may also be thus obtained, but it seems undesirable to risk extensive incisions for the purpose.

Prostate. This organ is not accessible to probatory incisions, and since the existence of carcinoma can be excluded only by examination of the whole gland, a negative report on a portion of tissue is of little value.

Breast. Mammary diseases in which a probatory incision through sound skin is indicated, are rare. When the question arises between chronic mastitis and carcinoma, if any incision is made, it is usually the safest procedure to remove the whole breast by Warren's plastic resection and submit the entire organ for gross examination. If no malignant process is found, one has merely removed a menace to the patient, since any chronic mastitis which has progressed so far as to suggest carcinoma frequently develops into carcinoma later. In women under thirty-five years with localized chronic induration of the breast, it is perhaps permissible to excise a portion of tissue for frozen section. I have known such a procedure to save the breast without subsequent recurrence of disease. In all such cases, however, it is safer to excise the entire suspected area. If the excised tissue proves to be carcinoma, it can hardly be doubted that the best surgical principles have been violated, but it is perhaps too much to assert that the patient's chances have been jeopardized if the probatory incision is immediately followed by radical operation. It is much more injudicious to remove a small portion of a diffusely indurated breast and base the subsequent procedure on the results of examination of a single piece of tissue. In chronic mastitis, carcinomatous areas are often multiple and difficult to detect. The practice of aspirating cysts for diagnosis is also hazardous. In women under thirty years a single cyst is usually unaccompanied by a malignant process, while after thirty or thirty-five years, carcinoma is often found in the cyst wall, or in the neighborhood, or it develops later. Hence in a woman of thirty-five years it is unsafe to base conclusions regarding the condition of the whole breast even on the study of an excised cyst wall. The conclusion therefore follows that at no age is the excision of a simple cyst a satisfactory procedure. The very variable circumstances under which tumors and chronic indurative diseases of the breast arise, render it impossible to apply rigid rules governing the probatory incision. Each case must be considered by itself.

Uterus. The uterus is perhaps the organ most frequently subjected to probatory incision and curettage, and the material consists of polyps, eroded cervices, and curettings. The removal of accessible polyps is accomplished without danger and often with curative results. The excision of a portion of a carcinomatous cervix, however, can hardly be treated as an inconsequential matter. Considerable crushing of tissues is usually inflicted in cutting deeply into the indurated cervix uteri, and unless the desired tissue can be obtained without undue manipulation it might better be dispensed with. There is much ground for attributing the high percentage of recurrence of cervical and corpus carcinoma to the mechanical dissemination of tumor cells during examination and operation. I have found tumor cells from a very small early superficial corpus carcinoma squeezed entirely through the lymphatics of the fundus by the trauma of hysterectomy. The disease recurred in the pelvic nodes. Although adenocarcinomatous polyps may be eradicated by curettage, and although diffuse adenocarcinoma may be strictly limited to the mucosa, energetic curettage would seem more likely to disseminate tumor cells into the lymphatics than to cure the disease. The practice of curettage for the treatment or diagnosis of corpus carcinoma must, on anatomical grounds, be regarded as distinctly dangerous. The very careful removal of a limited portion of the suspected endometrium is all that can be approved, and it may well be that the cervical dilatation required to reach the fundus is itself a distinct hazard.

Lymph nodes. In systemic diseases of the lymphatic system, the excision of a lymph node is usually the only safe method of diagnosis and cannot be regarded as disseminating the disease. It may sometimes be avoided by previous examination of the blood. On the other hand, the practice of removing enlarged lymph nodes for the diagnosis or prognosis of carcinomas of adjoining mucous membranes must be deprecated or employed as a last resort. If the node is involved, no good has been accomplished and the site of the disease must still be determined. If the node is not involved, a barrier to dissemination has been removed and trauma inflicted, while the diagnosis still remains undetermined.

Sarcoma of bones. Incision through the intact skin to remove portions of suspected tumors of bone or fascia frequently carries infection, often fails to reach the tumor tissue, may aggravate the tumor process through trauma, and sometimes leads to erroneous conclusions. Chiselling off portions of periosteum and hard bone in suspected sarcoma is especially to be deprecated. Only a very competent operator, fully acquainted with the gross anatomy of bone sarcoma, should undertake such incisions. With fungating sarcomas the excision of a portion of tumor tissue is usually accomplished with precision.

In general, the attitude regarding probatory incision of tumors should be conservative. The resort to this method of diagnosis is a confession of ignorance. The extent to which it must be employed depends on the experience and diagnostic capacity of the observer. It is possible by long training to recognize the nature of most accessible tumors by various clinical signs, and the hasty resort to microscopic diagnosis tends to

hamper the development of other diagnostic methods and of general clinical judgment. In not a few instances the clinical signs are more specific than the microscopical structure of a tissue section. The microscope should be employed, therefore, only after other means have failed.

Having failed by other methods to establish a diagnosis, the wisdom of resorting to probatory incision must be determined for each particular case. There will always remain a large number of conditions in which the fullest possible clinical analysis leaves doubt of the nature of the disease, and when important variations in treatment may depend on positive diagnosis, the microscopic evidence must be invoked. The wisdom of removing tissues for diagnosis may often depend on the possibility of ecuring a competent pathological report. The assumption that any laboratory tyro who cuts sections in a chemical, bacterial, or commercial laboratory, or a drug store, is a competent judge of histopathology, appears to be widely held. Yet any surgeon who fails to investigate the technical standards of the laboratory where he sends material and the competency of the examiner who passes upon it, fails in his duty to his patient. I believe that all general pathologists will support the assertion that the histological interpretation of tumors is a very extensive and difficult medical specialty, requiring a mature knowledge of general pathology and a long practical experience with tumor tissues.

Finally, the restrictions here suggested in the use of probatory excisions of tumors do not in any way apply to the thorough study of the tumor after operation. With the whole material in hand it is difficult to place any limit to the scope of the examination desirable or to the importance of the conclusions thus reached in determining the diagnosis, prognosis and further treatment of the case. It cannot be too strongly emphasized that the scope of this study must include, not only the microscopical structure, but also the gross anatomical features and the complete clinical record.

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